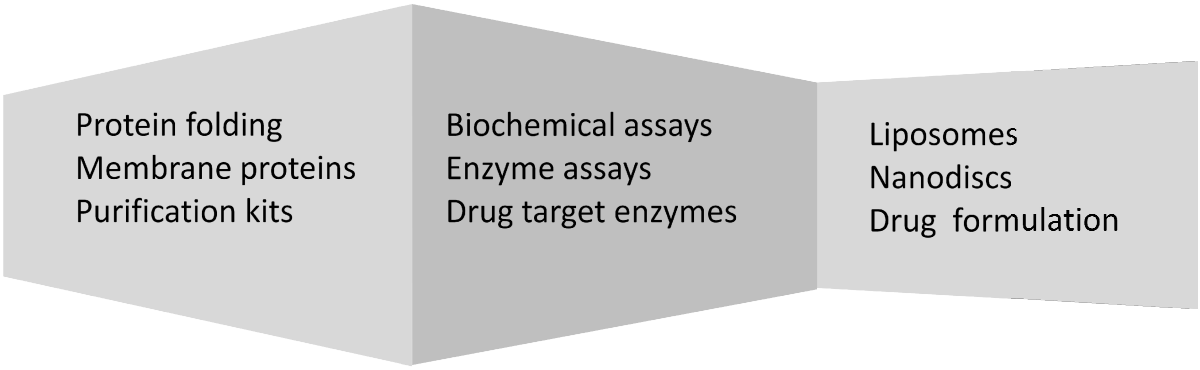
ProFoldin

Products and Services for Life Science and Drug Discovery



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Products and services overview

ProFoldin provides innovative and unique research tools for life sciences and drug discovery, focusing on the following areas:

- Drug target enzyme assays
- Concentration measurement of various molecules and ions
- Liposome and nanodisc products
- Protein folding, extraction and reconstitution tools
- Purification tools
- Molecular binding plates

The biochemical assays and drug target assays are based on light absorbance or fluorescence detections. The assays are high throughput and suitable for HTS applications. ProFoldin's products are generated with innovations in biochemistry, protein science and nanotechnologies. The products are broadly employed by universities, research institutes, biotech companies, pharmaceutical R&D and research hospitals worldwide in the communities of life sciences and drug discovery.

ProFoldin also offers assay services including custom assay development, enzyme activity tests, screening of compound libraries and IC50 measurements.

Contents

Chapter 1

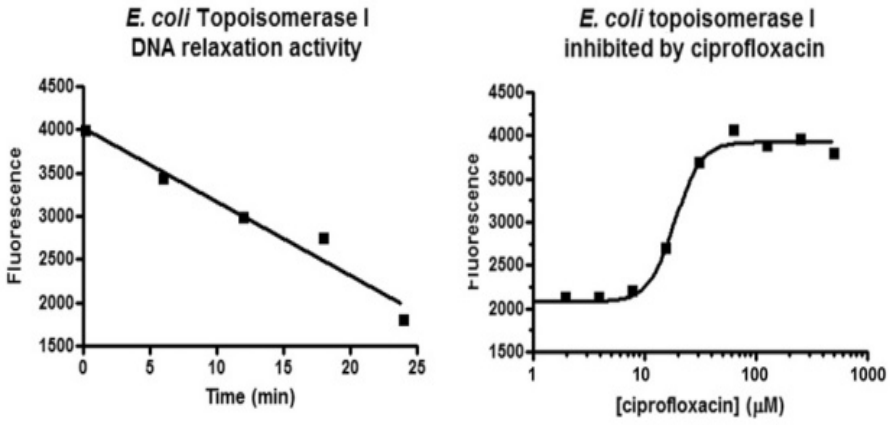
Drug target and enzyme assays

Various high throughput enzyme assays are available for academic research of drug target proteins and tests of enzyme inhibitors from compound libraries. These assays are in a 96-well or 384-well plate format and based on detection of light absorbance or fluorescence signals. The assays are rapid. They are not-radioactive. No tedious gel electrophoresis or HPLC process is needed. The validated drug targets are enzymes in various biological systems including DNA replication, gene transcription, gene regulation, cell membrane synthesis and other metabolism process which are essential for growth of bacterial cells, cancer cells or virus.

1.1. DNA Topoisomerase assays

1.1.1 DNA relaxation assays

DNA topoisomerases such as bacterial topoisomerase I convert supercoiled circular DNA into relaxed DNA (DNA relaxation reaction). Bacterial topoisomerase IV also has the DNA relaxation activity. The DNA Topoisomerase I Assay is based on the principle that the relaxed DNA suppresses the fluorescent intensity much more than the supercoiled DNA when the DNA interacts with fluorescence dye H19 (a fluorescence dye for DNA relaxation / supercoiling assay) in the presence of magnesium. When the supercoiled DNA is converted into its relaxed form, the fluorescent signal decreases.



1.1.1.1 Human DNA topoisomerase I

Human Topoisomerase I DNA Relaxation Assay Kit Plus -100 (Catalog No. HRA100KE)

Kit components: includes 480 µl of 10 x Buffer HT1, 405 µl of 10 x supercoiled plasmid DNA, 10 µl of 5000 x human topoisomerase I enzyme (500,000 U/ml), 20 µl of 1500 x Dye H19 and 3000 µl of 10 x H19 dilution buffer. It includes all the reagents for 100 DNA relaxation assays in a 96-well plate format or 200 assays in a 384-well assay format.

1.1.1.2 Bacterial DNA topoisomerase I

***E. coli* DNA Topoisomerase I Assay Kit Plus-100 (Catalog No. DRA100KE)**

Kit components: 480 µl 10 x Buffer T1, 405 µl 10 x supercoiled DNA, 20 µl 1500 x Dye H19, 3000 µl 10 x H19 dilution buffer, 42 µl 100 x *E coli* Topo I.

1.1.1.3 DNA relaxation by other DNA topoisomerases

***E. coli* Topo IV DNA Relaxation Assay Kit Plus (Catalog No. T4RA-100KE)**

Kit components: 480 µl 10 x Buffer T2, 405 µl 10 x supercoiled DNA, 20 µl 1500 x H19 dye, 3 ml 10 x H19 dilution buffer, 420 µl 10 mM ATP, 45 µl 100x *E.coli* Topo IV.

***P. aeruginosa* Topo IV DNA Relaxation Assay Kit Plus (Catalog No. T4RA-100KP)**

Kit components: 480 µl 10 x Buffer T2, 405 µl 10 x supercoiled DNA, 20 µl 1500 x H19 dye, 3 ml 10 x H19 dilution buffer, 420 µl 10 mM ATP, 43 µl 100x *P. aeruginosa* Topo IV.

***S. aureus* Topo IV DNA Relaxation Assay Kit Plus (Catalog No. T4RA-100KS)**

Kit components: 480 µl 10 x Buffer T2, 405 µl 10 x supercoiled DNA, 20 µl 1500 x H19 dye, 3 ml 10 x H19 dilution buffer, 420 µl 10 mM ATP, 43 µl 100 x *S. aureus* Topo IV.

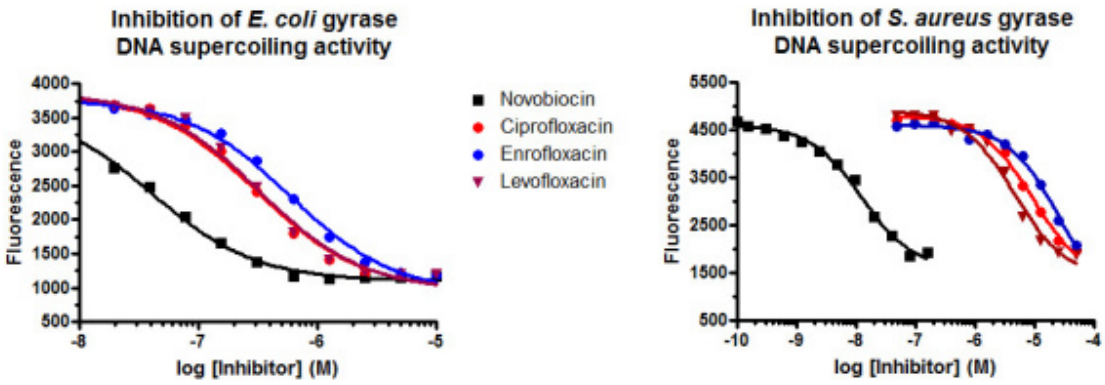
***S. pneumoniae* Topo IV DNA Relaxation Assay Kit Plus (Catalog No. T4RA-100KN)**

Kit components: 480 µl 10 x Buffer T2, 405 µl 10 x supercoiled DNA, 20 µl 1500 x H19 dye, 3 ml 10 x H19 dilution buffer, 420 µl 10 mM ATP, 43 µl 100x *S. pneumoniae* Topo IV.

1.1.2 Gyrase DNA supercoiling assays

DNA topoisomerases such as bacterial topoisomerase II (gyrase) convert relaxed circular DNA into supercoiled DNA (DNA supercoiling reaction). The DNA Topoisomerase II (Gyrase) Assay is based on the principle that the supercoiled DNA and relaxed DNA yield different fluorescent

intensity when interact with fluorescence dye H19. The relaxed DNA suppresses the fluorescent intensity much more than the supercoiled DNA in the presence of magnesium. Therefore, when the relaxed DNA is converted into its supercoiled form, the fluorescent signal increases. The change of fluorescence intensity is used to measure the supercoiling reaction of gyrases and high throughput screen of gyrase inhibitors.



***E. coli* DNA Topoisomerase II (Gyrase) Assay Kit Plus-100 (Catalog No. DSA100KE)**

Kit components: 600 µl 10 x Buffer T2, 405 µl 10 x relaxed DNA, 20 µl 1500 x H19 dye, 450 µl 10 x ATP, 3000 µl 10 x H19 dilution buffer, 50 µl 100 x *E.coli* gyrase. It is for 100 assays in a 96-well plate format.

***S. aureus* Gyrase DNA Supercoiling Assay Kit Plus (Catalog No. DSA020KSE)**

Kit components: 160 µl 10 x Buffer T2, 180 µl 2 M KGlu, 84 µl 10 x relaxed DNA, 6 µl 1500 x H19 dye, 90 µl 10 x ATP, 550 µl 10 x H19 dilution buffer, 10 µl 100 x *S. aureus* gyrase. It is for 100 assays in a 96-well plate format.

***S. aureus* Gyrase DNA Supercoiling Assay Plus-100 (Catalog No. DSA100KSE)**

Kit components: 500 µl 10 x Buffer T2, 820 µl 2 M KGlu, 405 µl 10 x relaxed DNA, 20 µl 1500 x H19 dye, 450 µl 10 x ATP, 3000 µl 10 x H19 dilution buffer, 43 µl 100 x *S. aureus* gyrase. It is for 100 assays in a 96-well plate format.

***P. aeruginosa* Gyrase DNA Supercoiling Assay Kit Plus (Catalog No. DSA020KPE)**

Kit components: 160 µl 10 x Buffer T2, 84 µl 10 x relaxed DNA, 6 µl 1500 x H19 dye, 90 µl 10 x ATP, 550 µl 10 x H19 dilution buffer, 12 µl 100 x *P. aeruginosa* gyrase. It is for 100 assays in a 96-well plate format.

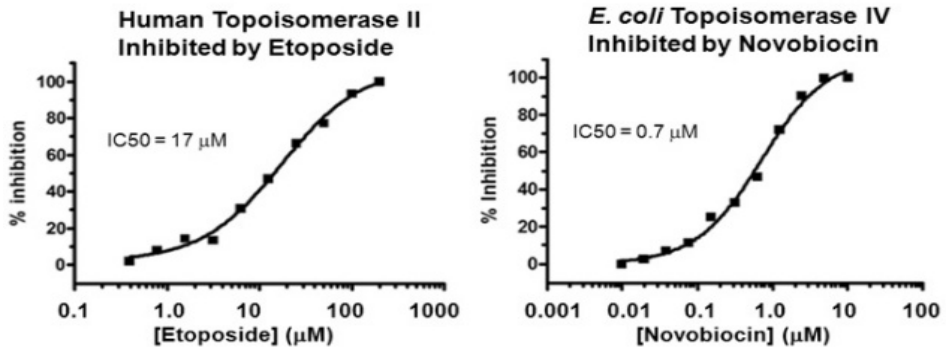
***P. aeruginosa* Gyrase DNA Supercoiling Assay Plus-100 (Catalog No. DSA100KPE)**
Kit components: 600 µl 10 x Buffer T2, 405 µl 10 x relaxed DNA, 20 µl 1500 x H19 dye, 450 µl 10 x ATP, 3000 µl 10 x H19 dilution buffer, 45 µl 100 x *P. aeruginosa* gyrase. It is for 100 assays in a 96-well plate format.

1.1.3 Topoisomerase DNA decatenation assays

DNA topoisomerases such as bacterial DNA topoisomerase IV (the parC-parE complex) and human topoisomerase II convert the concatenated DNA into decatenated DNA during the DNA replication process in the cell. DNA decatenation activity by other topoisomerases including topoisomerase I and bacterial gyrases are also observed.

The **Spin-column DNA Topoisomerase Decatenation Assays** are based on the principle that the decatenated DNA is separated from the concatenated DNA by a quick and easy spin-column process. The concatenated DNA stays on the column, while the decatenated DNA is eluted. The eluted DNA is quantified by fluorescence.

The **96-Well Topoisomerase DNA Decatenation Assay** is in a 96-well assay plate format that can be used for high-throughput tests of topoisomerase inhibitors. The assay is based on the principle that the decatenated DNA is separated from the concatenated DNA by a filtration process. The decatenated DNA passed through the filter (TDD filter plate), received in a black 96 well plate and quantified by fluorescence at 535 nm (excitation at 485 nm).



1.1.3.1 Bacterial topoisomerase IV

***E. coli* DNA Topoisomerase IV Assay Kit Plus-100 (Catalog No. DDC100KE)**

Kit components: 400 μl 10 x Buffer T4, 255 μl 10 x concatenated DNA, 780 μl 20 x Dye, 550 μl 0.5 M EDTA, 100 spin columns, 120 μl 10 mM ATP, 55 μl 100 *x E.coli* topo IV. It is for 100 assays in a 96-well plate format.

96-Well *E. coli* Topo IV DNA Decatenation Assay Kit Plus (Catalog No. EDD96KE)

Kit components: 600 μl 10 x Buffer T2, 50 μl 10x concatenated DNA, 530 μl 10 x fluorescence dye, 1 TDD plate,1 V-bottom plate, 2 ml 10 x Rinse buffer, 8 μl 1000 x *E. coli* topo IV, 120 μl 10 mM ATP, 500 μl enzyme dilution buffer, 1 ml 0.4 M EDTA, 1 receiver plate. It is for 96 assays in a 96-well plate format.

96-Well Topoisomerase DNA Decatenation Assay Kit (Catalog No. TDD96K)

Kit components: 600 μl 10 x Buffer T2, 500 μl 10x concatenated DNA, 260 μl 20 x fluorescence dye, 1 TDD plate, 2 ml 10 x Rinse buffer, 110 μl 10 mM ATP, 1 receiver plate, 1 V-bottom plate. It is for 96 assays in a 96-well plate format.

96-Well *P. aeruginosa* Topo IV DNA Decatenation Assay Kit Plus (Catalog No. PDD96KE)

Kit components: 600 μl 10 x Buffer T2, 500 μl 10x concatenated DNA, 530 μl 10 x fluorescence dye, 1 TDD plate,1 V-bottom plate, 2 ml 10 x Rinse buffer, 8 μl 1000 x *P. aeruginosa* topo IV, 120 μl 10 mM ATP, 500 μl enzyme dilution buffer, 1 ml 0.4 M EDTA, 1 receiver plate. It is for 96 assays in a 96-well plate format.

96-Well *S. aureus* Topo IV DNA Decatenation Assay Kit Plus (Catalog No. SDD96KE)

Kit components: 600 μl 10 x Buffer T2, 500 μl 10x concatenated DNA, 530 μl 10 x fluorescence dye, 1 TDD plate,1 V-bottom plate, 2 ml 10 x Rinse buffer, 8 μl 1000 x *S. aureus* topo IV, 120 μl 10 mM ATP, 500 μl enzyme dilution buffer, 1 ml 0.4 M EDTA, 1 receiver plate. It is for 96 assays in a 96-well plate format.

96-Well *S. pneumoniae* Topo IV DNA Decatenation Assay Kit Plus (Catalog No. NDD96KE)

Kit components: 600 μl 10 x Buffer T2, 500 μl 10x concatenated DNA, 530 μl 10 x fluorescence dye, 1 TDD plate,1 V-bottom plate, 2 ml 10 x Rinse buffer, 8 μl 1000 x *S. pneumoniae* topo IV,

120 μl 10 mM ATP, 500 μl enzyme dilution buffer, 1 ml 0.4 M EDTA, 1 receiver plate. It is for 96 assays in a 96-well plate format.

1.1.3.2 Human DNA topoisomerase II

Human Topoisomerase II DNA Decatenation Assay Kit Plus-100 (Catalog No. HDC100KE)

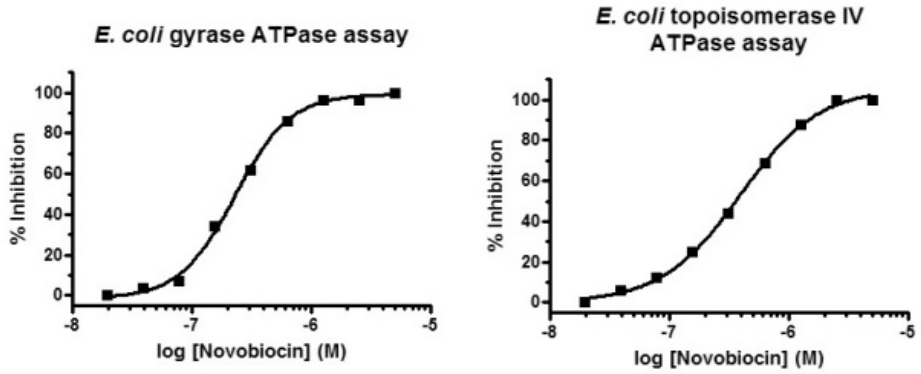
Kit components: 550 μl 10 x Buffer HT, 520 μl 10 x concatenated DNA, 800 μl 20 x Dye, 600 μl 0.5 M EDTA, 100 spin columns, 120 μl 10 mM ATP, 7.5 μl 10 U/ml human topo II, 1000 μl enzyme dilution buffer. It is for 100 assays in a spin-column assay format.

96-Well Human Topo II DNA Decatenation Assay Kit Plus (Catalog No. HDD96KE)

Kit components: 600 μl 10 x Buffer T2, 500 μl 10x concatenated DNA, 530 μl 10 x fluorescence dye, 1 TDD plate, 1 V-bottom plate, 2 ml 10 x Rinse buffer, 6 μl 1000x human topo II, 120 μl 10 mM ATP, 500 μl enzyme dilution buffer, 1 ml 0.4 M EDTA, 1 receiver plate. It is for 96 assays in a 96-well plate format.

1.1.4 Topoisomerase ATPase assays

Type II DNA topoisomerases including topoisomerase II and topoisomerase IV hydrolyze ATP as the source of molecular energy to carry out DNA supercoiling and DNA decatenation reactions. Inhibition of the ATPase activity of topoisomerases by compounds such as novobiocin blocks their biological functions. Therefore, the topoisomerase ATPase assay can be used for high-throughput screen of topoisomerase inhibitors. The Topoisomerase ATPase Assay is based on detection of the phosphate produced by the ATP hydrolysis reaction in the presence of DNA. The assay is in a 384-well plate format and the phosphate is detected using light absorbance at 650 nm.



***E. coli* gyrase ATPase assay Kit Plus (Catalog No. T2A-100KE)**

Kit components: 400 μl 10 x Buffer T2, 35 μl 100 x DNA, 35 μl 1000x ATP (20 mM), 33 μl 100 x topoisomerase IV, 5 ml Dye. It is for 100 assays in a 384-well plate format.

Topoisomerase ATPase Assay Kit (Catalog No. TA1000)

Kit components: 4 ml 10 x Buffer T2, 320 μl 100 x DNA, 320 μl 100 x ATP (20 mM), 50 ml Dye. It is for 1000 assays in a 384-well plate format.

***S. aureus* gyrase ATPase assay Kit Plus (Catalog No. T2A-100KS)**

Kit components: 400 μl 10 x Buffer T2, 35 μl 100 x DNA, 35 μl 1000x ATP (20 mM), 33 μl 100 x *S. aureus* topoisomerase IV, 5 ml Dye. It is for 100 assays in a 384-well plate format.

***E. coli* Topo IV ATPase Assay Kit Plus (Catalog No. T4A-100KE)**

Kit components: 400 μl 10 x Buffer T2, 35 μl 100 x DNA, 35 μl 1000 x ATP (20 mM), 33 μl 100 x *E. coli* topoisomerase IV, 5 ml Dye. It is for 100 assays in a 384-well plate format.

***S. pneumoniae* Topo IV ATPase Assay Kit Plus (Catalog No. T4A-100KN)**

Kit components: 400 μl 10 x Buffer T2, 35 μl 100 x DNA, 35 μl 1000x ATP (20 mM), 33 μl 100 x *S. pneumoniae* topoisomerase IV, 5 ml Dye. It is for 100 assays in a 384-well plate format.

***P. aeruginosa* Topoisomerase IV ATPase Assay Kit Plus (Catalog No. T4A-100KP)**

Kit components: 400 μl 10 x Buffer T2, 35 μl 100 x DNA, 35 μl 1000x ATP (20 mM), 33 μl 100 x *P. aeruginosa* topoisomerase IV, 5 ml Dye. It is for 100 assays in a 384-well plate format.

***S. aureus* Topoisomerase IV ATPase Assay Kit Plus (Catalog No. T4A-100KS)**

Kit components: 400 μl 10 x Buffer T2, 35 μl 100 x DNA, 35 μl 1000x ATP (20 mM), 33 μl 100 *x S. aureus* topoisomerase IV, 5 ml Dye. It is for 100 assays in a 384-well plate format.

1.1.5 Topoisomerase assay substrates

The relaxed plasmid DNA is a substrate of DNA supercoiling enzymes such as bacterial gyrases. The supercoiled DNA is a substrate of DNA relaxation enzymes such as DNA topoisomerase I and topoisomerase IV. The DNA supercoiling and relaxation reactions can be monitored by fluorescence dye H19 based on the principle that the supercoiled DNA and relaxed DNA yield different fluorescent intensity when the DNA interacts with fluorescence dye H19.

Relaxed plasmid DNA - 0.050 mg (Catalog No. RDNA-050UG)
Kit components: 50 μl 1 mg/ml relaxed DNA.

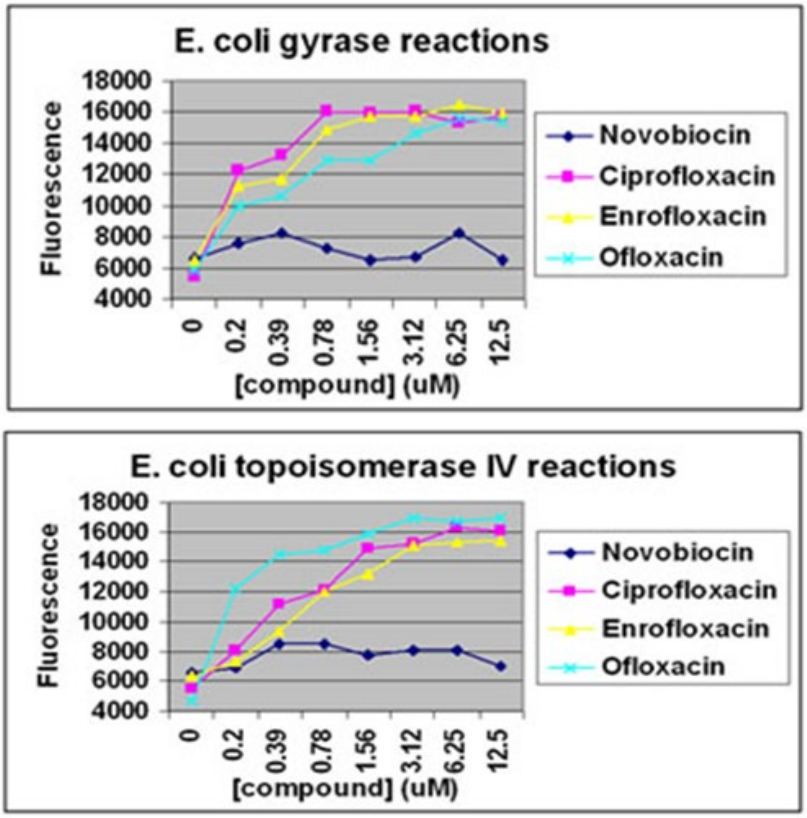
Relaxed plasmid DNA - 1 mg (Catalog No. RDNA-1MG)
Kit components: 1000 μl 1 mg/ml relaxed DNA.

Supercoiled plasmid DNA - 0.050 mg (Catalog No. SDNA-050UG)
Kit components: 50 μl 1 mg/ml supercoiled DNA.

Supercoiled plasmid DNA - 1 mg (Catalog No. SDNA-1MG)
Kit components: 1000 μl 1 mg/ml supercoiled DNA.

1.1.6 Topoisomerase DNA cleavage assays

Bacterial gyrase, topoisomerase IV (parC-parE) and mammalian topoisomerase II are type II topoisomerases. These topoisomerases change DNA topology by cleavage and religation of both DNA strands. When an inhibitor such as a fluoroquinolone drug blocks the religation step of the topoisomerase reaction, the covalent complex between the cleaved or linearized DNA and the topoisomerase (cleavage complex) is accumulated. Formation of the DNA cleavage complex causes DNA damage in the cells (topoisomerase poison). Testing formation of DNA cleavage complexes is useful for understanding the topoisomerase inhibition mechanism of novel topoisomerase inhibitors. The 96-well topoisomerase DNA cleavage assay kits are based on the principle that the linearized DNA has much greater permeability through a solution matrix and a filter than the closed circular DNA. For example, both novobiocin and ciprofloxacin inhibit DNA gyrase. However, novobiocin does not cause formation of cleavage complex. Ciprofloxacin does. The assay kit is in 96-well plate format and can be used for testing topoisomerase poison compounds in a high throughput setting.



96-well *E. coli* Gyrase DNA Cleavage Assay Kit Plus (Catalog No. T2C96KE)

Kit components: 300 µl 10 x TDC buffer, 30 µl 100 x DNA, 260 µl SDS solution, 260 µl Protease K, 10 ml TDC matrix, 6 ml Rinse buffer, 500 µl 10 x TDC dye, 1 TDC filter plate, 30 µl 100 mM ATP, 1 black plate, 1 V-bottom plate, 26 µl 100 x *E. coli* gyrase. It is for 96 assays in a 96-well plate format.

96-well Topoisomerase DNA Cleavage Assay Kit (enzyme not included) Catalog No. TDC96K)

Kit components: 300 µl 10 x TDC buffer, 30 µl 100 x DNA, 260 µl SDS solution, 260 µl Protease K, 10 ml TDC matrix, 6 ml Rinse buffer, 500 µl 10 x TDC dye, 1 TDC filter plate, 30 µl 100 mM ATP, 1 black plate, 1 V-bottom plate. It is for 96 assays in a 96-well plate format.

96-well *E. coli* Topo IV DNA Cleavage Assay Kit Plus (Catalog No. T4C96KE)

Kit components: 300 µl 10 x TDC buffer, 30 µl 100 x DNA, 260 µl SDS solution, 260 µl Protease K, 10 ml TDC matrix, 6 ml Rinse buffer, 500 µl 10 x TDC dye, 1 TDC filter plate, 30 µl 100 mM ATP,

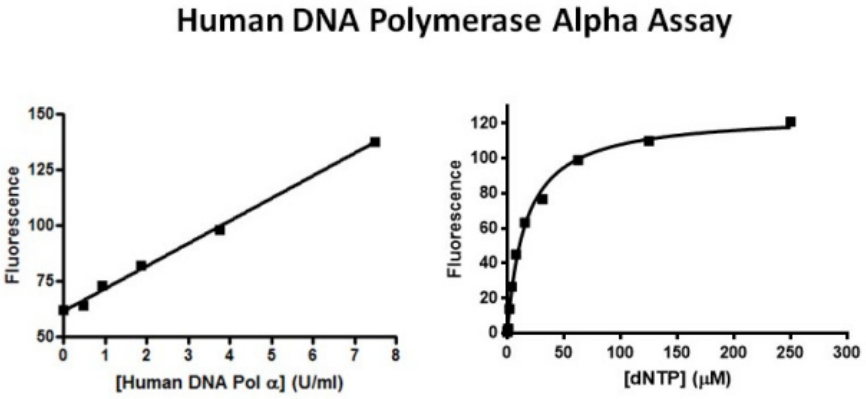
1 black plate, 1 V-bottom plate, 26 µl 100 x *E. coli* topoisomerase IV. It is for 96 assays in a 96-well plate format.

96-well *S. aureus* Topo IV DNA Cleavage Assay Kit Plus (Catalog No. T4C96KS)
Kit components: 300 µl 10 x TDC buffer, 30 µl 100 x DNA, 260 µl SDS solution, 260 µl Protease K, 10 ml TDC matrix, 6 ml Rinse buffer, 500 µl 10 x TDC dye, 1 TDC filter plate, 30 µl 100 mM ATP, 1 black plate, 1 V-bottom plate, 26 µl 100 x *S. aureus* topoisomerase IV. It is for 96 assays in a 96-well plate format.

1.2 DNA polymerase assays

1.2.1 Human DNA polymerase alpha

Human DNA Polymerase Alpha (Pol α) is responsible for the initiation of DNA replication at origins of replication The Pol α enzyme consists of four subunits: the catalytic subunit POLA1, the regulatory subunit POLA2, and the small and the large primase subunits PRIM1 and PRIM2. The human DNA polymerase alpha assay is based on measurement of the DNA molecules synthesized by the DNA polymerase. The assay is performed in a 384-well or 96-well plate format. The assay can be used for detection of DNA polymerase alpha activity and high throughput screen of human DNA polymerase alpha inhibitors.

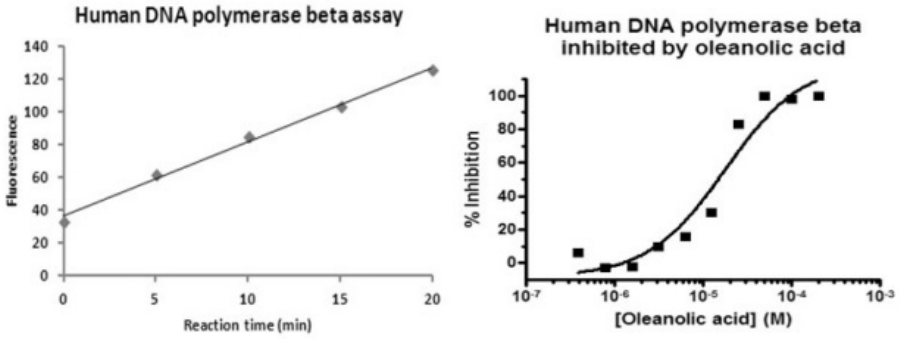


The **Human DNA Polymerase Alpha Assay Kit (Catalog No. HDPA100K)** includes all the assay kit components except the enzyme for 100 assays in a 384-well plate assay format: 400 µl of 10 x Buffer, 33 µl of 100 x DNA template, 33 µl of 100 x dNTP mix, 1550 µl of 2 x Dye and 1550 µl of 50 mM EDTA. The kit does not include the enzyme.

The **Human DNA Polymerase Alpha Assay Kit Plus (Catalog No. HDPA100KE)** includes all the assay kit components for 100 assays in 384-well plate assay format: 400 µl of 10 x Buffer, 33 µl of 100 x DNA template, 33 µl of 100 x dNTP mix, 10 µl of 300 x human DNA polymerase alpha, 1550 µl of 2 x Dye and 1550 µl of 50 mM EDTA.

1.2.2 Human DNA polymerase beta

DNA polymerase beta play key roles in the base excision repair (BER) process. It fills the small gaps (1 – 6 bases) of one DNA stand using the complementary strand as a template. Human DNA polymerase beta is an anti-cancer target. The human DNA polymerase beta assay is based on the principle that the repaired DNA forms more stable DNA duplex than the un-repaired one. The Assay Kit is to measure the formation of the repaired DNA by its fluorescence signal in the presence of a fluorescence dye.



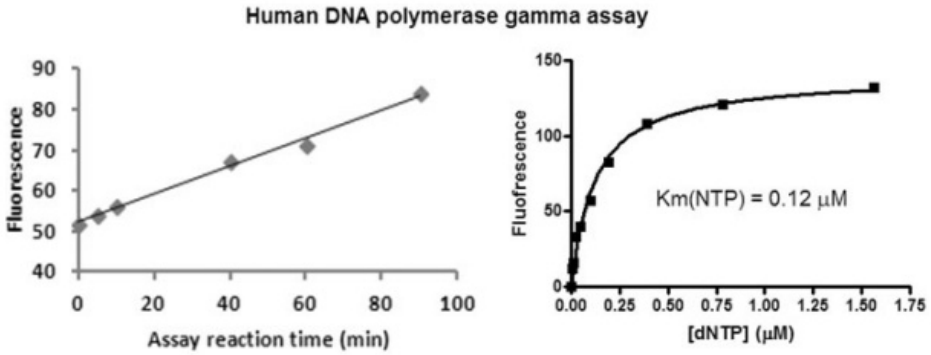
Each **Human DNA Polymerase Beta Assay Kit (Catalog No: DPB100K)** includes 800 µl of 10 x Buffer BP, 55 µl of 100x DNA template, 55 µl of 100 x dNTP mix, 22 ml of Reagent U and 420 µl of 10 x fluorescence dye for 100 assays of human DNA polymerase reactions in a 96-well plate format. The kit does not include human DNA polymerase beta.

Each **Human DNA Polymerase Beta assay Kit Plus (Catalog No: DPB100KE)** includes 800 µl of 10 x Buffer BP, 55 µl of 100x DNA template, 55 µl of 100 x dNTP mix, 22 ml of Reagent U, 52 µl of 100 x human DNA polymerase beta and 420 µl of 10 x fluorescence dye for 100 assays of human DNA polymerase reactions in a 96-well plate format.

1.2.3 DNA polymerase gamma

Human DNA polymerase gamma is the only DNA polymerase in human mitochondria and is responsible for the DNA replication, recombination and repairing in human mitochondria. It plays critical roles in mitochondrial diseases and aging. Some antiviral nucleoside analogs were

reported to inhibit DNA polymerase gamma after intracellular phosphorylation and cause severe chronic toxicity. The human DNA polymerase gamma assay is based on measurement of the DNA molecules synthesized by the DNA polymerase. The assay is performed in a 384-well or 96-well plate format. The assay can be used for detection of DNA polymerase gamma activity and high throughput screen of human DNA polymerase gamma inhibitors.

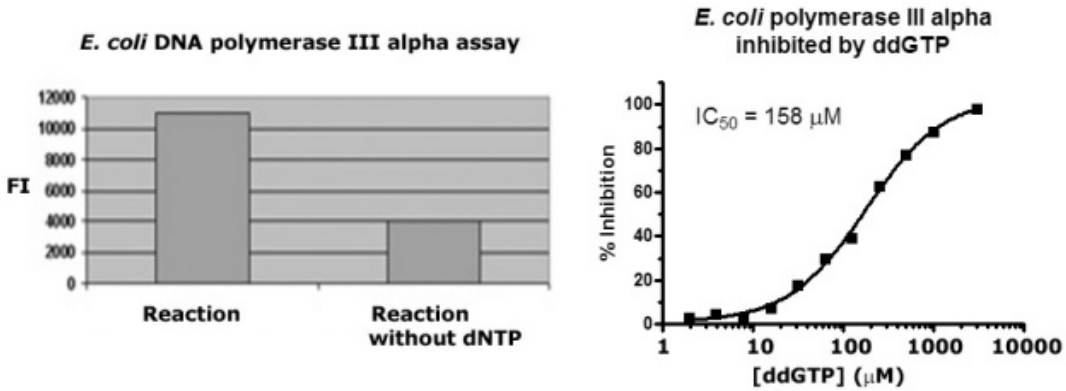


The **Human DNA Polymerase Gamma Assay Kit (Catalog No. DPG100K)** includes all the assay kit components except the enzyme for 100 assays in a 384-well plate assay format: 400 µl of 10 x Buffer, 33 µl of 100 x DNA template, 33 µl of 100 x dNTP mix, 1550 µl of 2 x Dye, 1550 µl of 50 mM EDTA.

The **Human DNA Polymerase Gamma Assay Kit Plus (Catalog No. DPG100KE)** includes all the assay kit components for 100 assays in 384-well plate assay format: 400 µl of 10 x Buffer, 33 µl of 100 x DNA template, 33 µl of 100 x dNTP mix, 33 µl of 100 x human DNA polymerase gamma, 1550 µl of 2 x Dye, 1550 µl of 50 mM EDTA.

1.2.4 Bacterial DNA polymerases

DNA polymerase III synthesizes DNA using the RNA primer made by the DNA primase at the DNA replication fork of bacteria. DNA polymerase III alpha is the catalytic subunit of the polymerase. The **DNA Polymerase Assay Kit** is based on measurement of the DNA molecules synthesized by the DNA polymerase. The assay can be performed in 96-well plate or 384-well plate format for high throughput screening of DNA polymerase inhibitors.



DNA Polymerase Assay Kit (enzyme not included) (Catalog No. DPA100K)
Each kit includes the assay reagents except the enzyme for 100 assays of DNA polymerase reactions in a 384-well assay format.

E. coli **DNA Polymerase Assay Kit Plus (enzyme included) (Catalog No. DPA100KE)**
Each kit includes all the assay reagents for 100 assays of DNA polymerase reactions in a 384-well assay format. *E. coli* DNA polymerase III alpha is included.

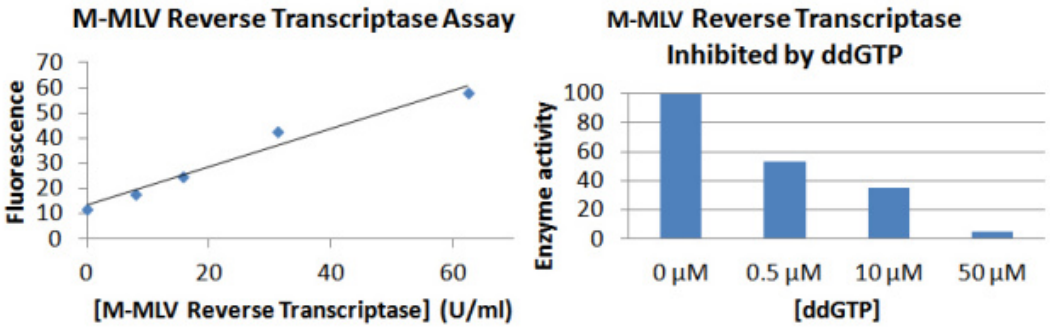
H. influenzae **DNA Polymerase Assay Kit Plus (enzyme included) (Catalog No. DPA100KH)**
Each kit includes all the assay reagents for 100 assays of DNA polymerase reactions in a 384-well assay format. *H. influenzae* DNA polymerase III alpha is included.

S. pneumoniae **DNA Polymerase Assay Kit Plus (enzyme included) (Catalog No. DPA100KN)**
Each kit includes all the assay reagents for 100 assays of DNA polymerase reactions in a 384-well assay format. *S. pneumoniae* DNA polymerase III alpha is included.

1.2.5 Virus reverse transcriptases

1.2.5.1 M-MLV reverse transcriptase

The Moloney murine leukemia virus (M-MLV) causes lymphocytic leukemia in mice. M-MLV reverse transcriptase (M-MLV RT) synthesizes a complementary DNA strand from single-stranded RNA or DNA. The M-MLV Reverse Transcriptase Assay is based on measurement of the DNA molecules synthesized by M-MLV RT. The assay can be performed in a 384-well or 96-well plate format for tests of M-MLV reverse transcriptase activities and high throughput screening of inhibitors.

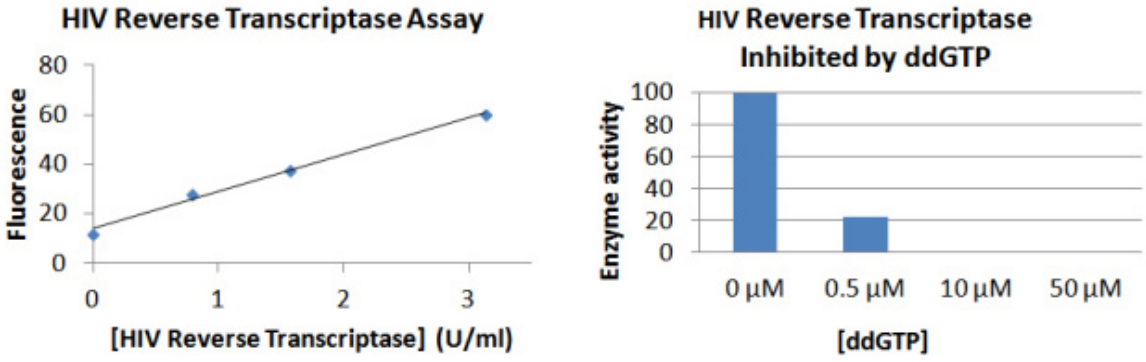


The **M-MLV Reverse Transcriptase Assay Kit (Catalog No: MLV100K)** includes 400 µl of 10 x Buffer, 33 µl of 100 x Template, 33 µl of 100 x dNTPs, 1550 µl of 2 x Dye and 1550 µl of 50 mM EDTA. It is for 100 assays of M-MLV Reverse Transcriptase in a 384-well plate format. The assay kit includes all reagents except the enzyme.

The **M-MLV Reverse Transcriptase Assay Plus(Catalog No: MLV100KE)** includes all reagents in the **M-MLV Reverse Transcriptase Assay Kit (Catalog No: MLV100K)** plus the enzyme, 7 µl 500 x M-MLV RT.

1.2.5.2 HIV reverse transcriptase

Infection of human immunodeficiency virus (HIV) causes acquired immunodeficiency syndrome (AIDS). The HIV reverse transcriptase (HIV RT) is an attractive drug target for HIV drug discovery. The HIV RT enzyme synthesizes a complementary DNA strand initiating from a primer using either RNA or single-stranded DNA as a template. The HIV Reverse Transcriptase Assay is based on measurement of the DNA molecules synthesized by HIV RT. The assay can be performed in a 384-well or 96-well plate format for tests of HIV reverse transcriptase activities and high throughput screening of inhibitors.

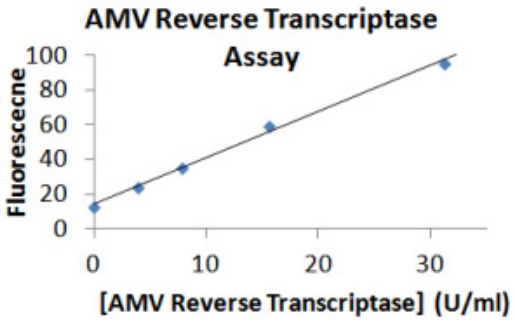


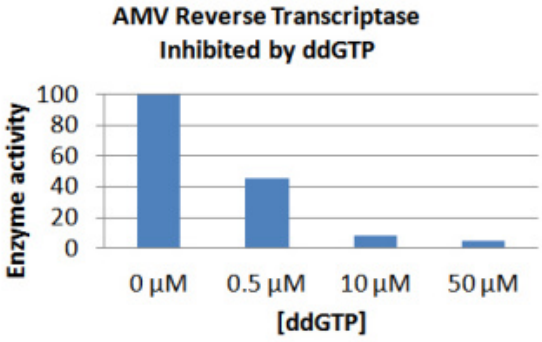
The **HIV Reverse Transcriptase Assay Kit (Catalog No: HIV100K**) includes 400 µl of 10 x Buffer, 33 µl of 100 x Template, 33 µl of 100 x dNTPs, 1550 µl of 2 x Dye and 1550 µl of 50 mM EDTA. It is for 100 assays of HIV Reverse Transcriptase in a 384-well plate format. The assay kit includes all reagents except the enzyme.

The **HIV Reverse Transcriptase Assay Plus (Catalog No: HIV100KE)** includes all reagents in the **HIV Reverse Transcriptase Assay Kit (Catalog No: HIV100K)** plus the enzyme, 7 µl 500 x HIV RT.

1.2.5.3 AMV reverse transcriptase

The avian myeloblastosis virus (AMV) is an alpha retrovirus responsible for acute myeloblastic leukemia (AML). AMV Reverse Transcriptase (AMV RT) catalyzes the synthesis of DNA using RNA or DNA template and dNTPs. The AMV Reverse Transcriptase Assay is based on measurement of the DNA molecules synthesized by AMV RT. The assay can be performed in a 384-well or 96-well plate format for tests of AMV reverse transcriptase activities and high throughput screening of inhibitors.





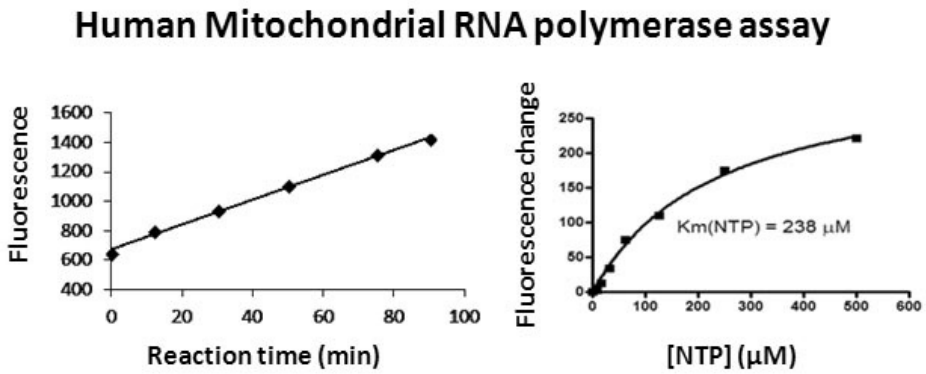
The **AMV Reverse Transcriptase Assay Kit (Catalog No: AMV100K)** includes 400 µl of 10 x Buffer, 33 µl of 100 x Template, 33 µl of 100 x dNTPs, 1550 µl of 2 x Dye and 1550 µl of 50 mM EDTA. It is for 100 assays of AMV Reverse Transcriptase in a 384-well plate format. The assay kit includes all reagents except the enzyme.

The **AMV Reverse Transcriptase Assay Plus (Catalog No: AMV100KE)** includes all reagents in the **AMV Reverse Transcriptase Assay Kit** (Catalog No: AMV100K) plus the enzyme, 7 µl 500 x AMV RT.

1.3 RNA polymerase assays

1.3.1 Human mitocondrial RNA polymerase

The human mitochondrial RNA polymerase (h-mtRNAP or POLRMT) functions as the transcriptase for expression and the primase for replication of mitochondrial DNA. Its malfunction is related to various diseases and aging. Some antiviral nucleoside analogs were reported to cause chronic toxicity and related to inhibition of mitochondrial RNA polymerase activity. The Human Mitochondrial RNA Polymerase Assay is based on measurement of the RNA molecules synthesized by the RNA polymerase using a DNA template and DNTPs. It is fluorescence assay in a 384-well or 96-well plate format. The assay can be used for measurement activities of human mitochondrial RNA polymerase and drug screens against this enzyme.



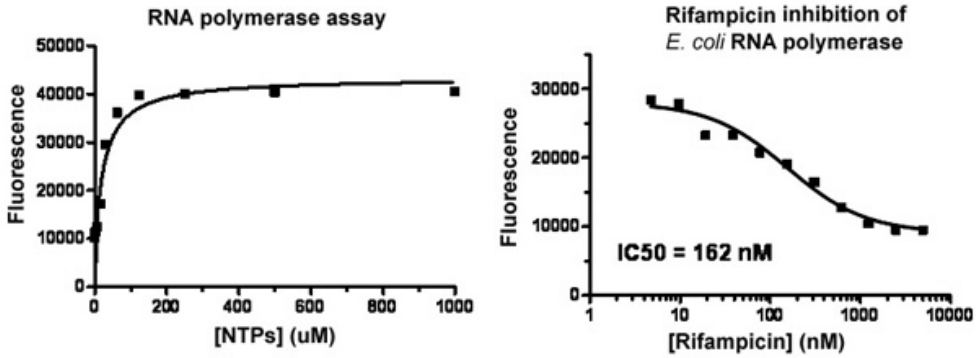
The **Human Mitochondrial RNA Polymerase Assay Kit (Catalog No. MRPA100K)** includes all the assay kit components except the enzyme for 100 assays in a 384-well plate assay format: 400 µl of 10 x Buffer, 33 µl of 100 x DNA template, 33 µl of 100 x NTP mix, 330 µl of 10 x fluorescence dye.

The **Human Mitochondrial RNA Polymerase Assay Kit Plus (Catalog No. MRPA100KE)** includes all the assay kit components for 100 assays in 384-well plate assay format: 400 µl of 10 x Buffer, 33 µl of 100 x DNA template, 33 µl of 100 x Human Mitochondrial RNA Polymerase, 33 µl of 100 x NTP mix, and 330 µl of 10 x fluorescence dye.

1.3.2 Bacterial RNA polymerases

The bacterial RNA polymerase is responsible for biosynthesis of mRNA, tRNA and rRNA in the cells. The **RNA Polymerase Assay Kit** is based on measurement of the RNA molecules

synthesized by the RNA polymerase using a single strand DNA template. The assay can be performed in 96-well plate or 384-well plate format for high throughput screening of RNA polymerase inhibitors. For bacterial RNAP assays using double strand DNA, please see RNAP pyrophosphate assays.



***E. coli* RNA Polymerase Assay Kit (enzyme not included) (Catalog No. RPA100K)**
Each kit includes the assay buffer and DNA template for 100 assays of RNA polymerase reactions in a 384-well assay format. The assay buffer is optimized for *E. coli* RNA polymerase. Enzyme is not included.

***E.coli* RNA polymerase Assay Kit Plus (enzyme included) (Catalog No. RPA100KE)**
The RNA polymerase assay kit including *E. coli* RNA polymerase.

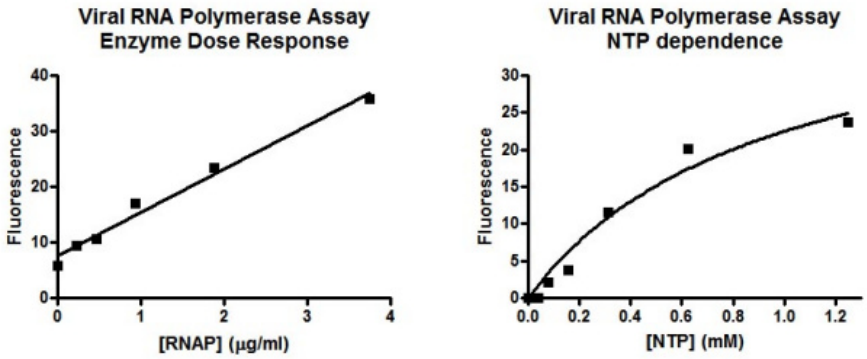
***S. aureus* RNA polymerase assay kit (enzyme not included) (RPA-100KS)**
Each kit includes the assay buffer and DNA template for 100 assays of RNA polymerase reactions in a 384-well assay format. The assay buffer is optimized for *S. aureus* RNA polymerase. Enzyme is not included.

***S. aureus* RNA polymerase assay kit Plus (enzyme included) (RPA-100KSE)**
The *S. aureus* RNA polymerase assay kit including *S. aureus* RNA polymerase.

1.3.3 Virus RNA polymerases

The Viral RNA-dependent RNA Polymerase Assay is developed using a RNA polymerase in the *Flaviviridae,* a family of positive, single-stranded, enveloped RNA viruses. The assay is based on measurement of the RNA molecules synthesized by the RNA polymerase using RNA as a template in the presence of NTPs. The assay can be performed in a 384-well or 96-well plate

format for tests of the enzyme activities of RNA polymerases in the *Flaviviridae* family and high throughput screening of inhibitors.

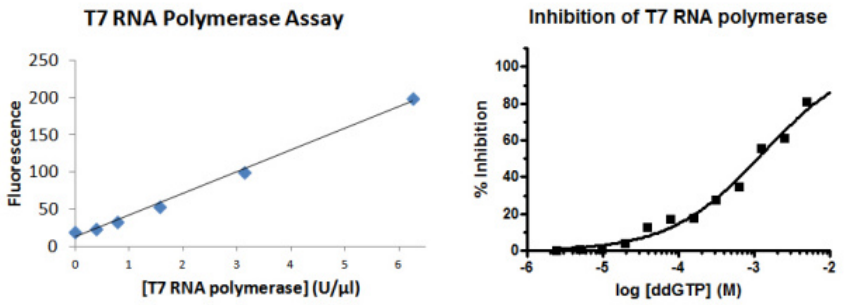


The **Viral RNA-dependent RNA Polymerase Assay Kit – 100 (Catalog No.VRT100K)** includes 350 µl of 10 x Buffer, 33 µl of 100 x Template, 33 µl of 100 x NTPs (50 mM ATP and 50 mM GTP) and 330 µl of 10 x fluorescence dye. It is for 100 assays of virus RNA polymerase in a 384-well plate format. The assay kit includes all reagents except the enzyme.

The **Viral RNA-dependent RNA Polymerase Assay Kit – 500** (**Catalog No.VRT500K)** includes 1550 µl of 10 x Buffer, 160 µl of 100 x Template, 160 µl of 100 x NTPs (50 mM ATP and 50 mM GTP) and 1550 µl of 10 x fluorescence dye. It is for 500 assays of virus RNA polymerase in a 384-well plate format. The assay kit includes all reagents except the enzyme.

1.3.4 Bacteriophage RNA polymerases

Bacteriophage T7 RNA polymerase specifically transcribes DNA downstream of a T7 promoter. The T7 RNA Polymerase Assay Kit is based on measurement of the RNA molecules synthesized by the RNA polymerase using a DNA template including a double strand T7 promoter. The assay can be performed in a 384-well plate format for tests of T7 RNA polymerase activities and high throughput screening of T7 RNA polymerase inhibitors.



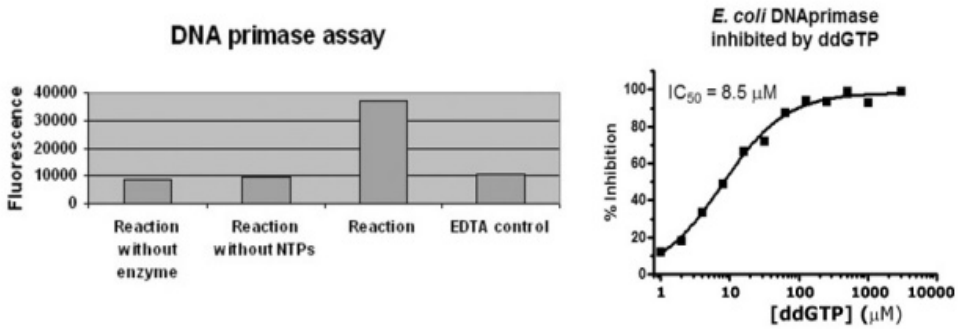
The **T7 RNA Polymerase Assay Kit (enzyme not included) (Catalog No T7RPA100K)** includes the assay buffer, DNA template, NTP mix and fluorescence dye for 100 assays of T7 RNA polymerase reactions in a 384-well assay format. The assay kit includes all reagents except the enzyme.

The **T7 RNA Polymerase Assay Kit Plus (enzyme included) (Catalog No T7RPA100KE)** includes the assay buffer, DNA template, NTP mix,T7 RNA polymerase and fluorescence dye for 100 assays of T7 RNA polymerase reactions in a 384-well assay format.

1.4 Assays for other DNA replication enzyme assays

1.4.1 DNA primase assays

The bacterial DNA primase (DnaG) synthesizes RNA primers at the DNA replication fork where the DNA helicase (DnaB) unwinds the double strand DNA. The **Bacterial DNA Primase Assays** are based on measurement of the RNA primers synthesized by the DNA primase in the presence of the DNA temperate and NTPS. DNA helicases dramatically stimulate the activities of gram-negative bacterial primases (e.g. *E. coli* and *H. influenzae*) but not gram-positive bacterial primases (*S. aureus* and *S. pneumoniae*). The assays can be performed in a 96-well plate or 384-well plate format for high throughput screening of DNA primase inhibitors.



***E. coli* DNA Primase Assay Kit (enzyme not included) (Catalog No. EGA100K)**

Kit components: 600 μl 10 x Buffer, 45 μl 100 x DNA, 850 μl 10 x fluorescence dye, 45 μl 100 x NTP. It is for 100 assays in a 96-well plate format.

***E. coli* DNA Primase Assay Kit Plus (*E. coli* primase-helicase included) (Catalog No. EGA100KE)**

Kit components: 600 μl 10 x Buffer, 45 μl 100 x DNA, 850 μl 10 x fluorescence dye, 45 μl 100 x NTP, 45 μl 100 x *E. coli* primase-helicase complex. It is for 100 assays in a 96-well plate format.

***E. coli* DNA Primase Assay Kit Plus-500 (*E. coli* primase-helicase included) (Catalog No. EGA500KE)**

Kit components: 2500 μl 10 x Buffer, 220 μl 100 x DNA, 4500 μl 10 x fluorescence dye, 220 μl 100 x NTP, 220 μl 100 x *E. coli* primase-helicase complex. It is for 500 assays in a 96-well plate format.

***H. influenzae* DNA Primase Assay Kit (enzyme not included) (Catalog No. HGA100K)**

Kit components: 600 μl 10 x Buffer, 45 μl 100 x DNA, 850 μl 10 x fluorescence dye, 45 μl 100 x NTP. It is for 100 assays in a 96-well plate format.

***H. influenzae* DNA Primase Assay Kit Plus (enzyme included) (Catalog No. HGA100KE)**

Kit components: 600 μl 10 x Buffer, 45 μl 100 x DNA, 850 μl 10 x fluorescence dye, 45 μl 100 x NTP, 45 μl 100 x *H.influenzae* primase-helicase complex. It is for 100 assays in a 96-well plate format.

***H. influenzae* DNA Primase Assay Kit Plus-500 (enzyme included) (Catalog No. HGA500KE)**

Kit components: 2500 μl 10 x Buffer, 220 μl 100 x DNA, 4500 μl 10 x fluorescence dye, 220 μl 100 x NTP, 220 μl 100 x *H.influenzae* primase-helicase complex. It is for 500 assays in a 96-well plate format.

***S. aureus* DNA Primase Assay Kit (enzyme not included) (Catalog No. AGA100K)**

Kit components: 600 μl 10 x Buffer, 45 μl 100 x DNA, 850 μl 10 x fluorescence dye, 45 μl 100 x NTP. It is for 100 assays in a 96-well plate format.

***S. aureus* DNA Primase Assay Kit Plus (enzyme included) (Catalog No. AGA100KE)**

Kit components: 600 μl 10 x Buffer, 45 μl 100 x DNA, 850 μl 10 x fluorescence dye, 45 μl 100 x NTP, 45 μl 100 x *S. aureus* primase. It is for 100 assays in a 96-well plate format.

***S. aureus* DNA Primase Assay Kit Plus-500 (enzyme included) (Catalog No. AGA500KE)**

Kit components: 2500 μl 10 x Buffer, 220 μl 100 x DNA, 4500 μl 10 x fluorescence dye, 220 μl 100 x NTP, 220 μl *S. aureus* 100x primase. It is for 500 assays in a 96-well plate format.

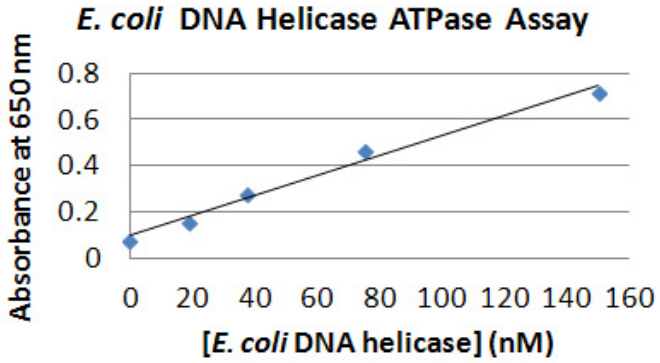
***S. pneumonia* DNA Primase Assay Kit (enzyme not included) (Catalog No. PGA100K)**
Kit components: 600 μl 10 x Buffer, 45 μl 100 x DNA, 850 μl 10 x fluorescence dye, 45 μl 100 x NTP. It is for 100 assays in a 96-well plate format.

***S. pneumonia* DNA Primase Assay Kit Plus (enzyme included) (Catalog No. PGA100KE)**
Kit components: 600 μl 10 x Buffer, 45 μl 100 x DNA, 850 μl 10 x fluorescence dye, 45 μl 100 x NTP, 45 μl 100 x *S. pneumoniae* primase. It is for 100 assays in a 96-well plate format.

***S. pneumonia* DNA Primase Assay Kit Plus-500 (enzyme included) (Catalog No. PGA500KE)**
Kit components: 2500 μl 10 x Buffer, 220 μl 100 x DNA, 4500 μl 10 x fluorescence dye, 220 μl 100 x NTP, 220 μl 100 x *S. pneumoniae* primase. It is for 500 assays in a 96-well plate format.

1.4.2 DNA helicase ATPase assays

DNA helicase (DnaB) hydrolyzes ATP as the source of molecular energy to carry out DNA unwinding required by the DNA replication process. Inhibition of the ATPase activity of DNA helicase blocks its DNA unwinding function. The DNA helicase ATPase assay can be used for high-throughput screen of DNA helicase inhibitors in drug discovery. The **DNA Helicase ATPase Assay** is based on detection of the phosphate produced by the ATP hydrolysis reaction in the presence of DNA. The assay is in a 384-well plate format and the phosphate is detected using light absorbance at 650 nm.



***E. coli* DNA Helicase ATPase assay kit Plus-100 (Catalog No. DNAB100KE)**

Kit components: 500 µl 10 x Buffer, 35 µl 100 x DNA, 65 µl 50 x *E. coli* Helicase, 35 µl 100 x ATP, 5 ml dye. It is for 100 assays in a 384-well plate format.

***E. coli* DNA Helicase ATPase assay kit Plus-500 (Catalog No. DNAB500KE)**
Kit components: 2 ml 10 x Buffer, 170 µl 100 x DNA, 320 µl 50 x *E. coli* Helicase, 170 µl 100 x ATP, 25 ml dye. It is for 500 assays in a 384-well plate format.

***P. aeruginosa* DNA Helicase ATPase assay kit Plus-100 (Catalog No. DNAB100KP)**
Kit components: 500 µl 10 x Buffer, 35 µl 100 x DNA, 65 µl 50 x *P. aeruginosa* Helicase, 35 µl 100 x ATP, 5 ml dye. It is for 100 assays in a 384-well plate format.

***P. aeruginosa* DNA Helicase ATPase assay kit Plus-500 (Catalog No. DNAB500KP)**
Kit components: 2 ml 10 x Buffer, 170 µl 100 x DNA, 320 µl 50 x *P. aeruginosa* Helicase, 170 µl 100 x ATP, 25 ml dye. It is for 500 assays in a 384-well plate format.

***H. influenzae* DNA Helicase ATPase assay kit Plus-100 (Catalog No. DNAB100KH)**
Kit components: 500 µl 10 x Buffer, 35 µl 100 x DNA, 65 µl 50 x *H. influenzae* Helicase, 35 µl 100 x ATP, 5 ml dye. It is for 100 assays in a 384-well plate format.

***H. influenzae* DNA Helicase ATPase assay kit Plus-500 (Catalog No. DNAB500KH)**
Kit components: 2 ml 10 x Buffer, 170 µl 100 x DNA, 320 µl 50 x *H influenzae* Helicase, 170 µl 100x ATP, 25 ml dye. It is for 500 assays in a 384-well plate format.

***S. pneumoniae* DNA Helicase ATPase assay kit Plus-100 (Catalog No. DNAB100KN)**
Kit components: 500 µl 10 x Buffer, 35 µl 100 x DNA, 65 µl 50 x *S. pneumoniae* Helicase, 35 µl 100x ATP, 5 ml dye. It is for 100 assays in a 384-well plate format.

***S. pneumoniae* DNA Helicase ATPase assay kit Plus-500 (Catalog No. DNAB500KN)**
Kit components: 2 ml 10 x Buffer, 170 µl 100 x DNA, 320 µl 50 x *S. pneumoniae* Helicase, 170 µl 100x ATP, 25 ml dye. It is for 500 assays in a 384-well plate format.

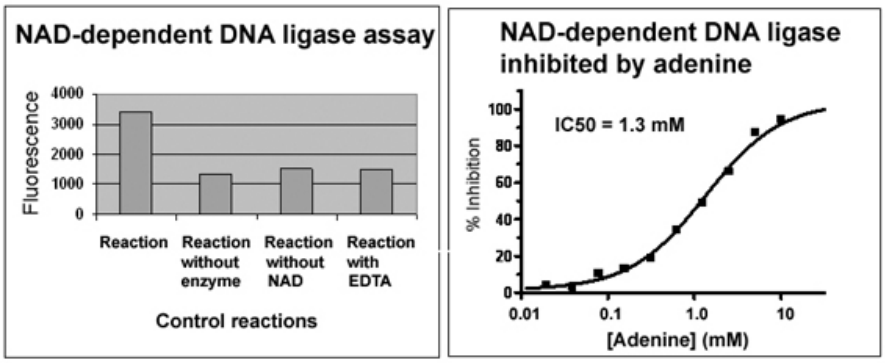
***S. aureus* DNA Helicase ATPase assay kit Plus-100 (Catalog No. DNAB100KS)**
Kit components: 500 µl 10 x Buffer, 35 µl 100 x DNA, 65 µl 50 x *S. aureus* Helicase, 35 µl 100x ATP, 5 ml dye. It is for 100 assays in a 384-well plate format.

***S. aureus* DNA Helicase ATPase assay kit Plus-500 (Catalog No. DNAB500KS)**

Kit components: 2 ml 10 x Buffer, 170 µl 100 x DNA, 320 µl 50 x *S. aureus* Helicase, 170 µl 100x ATP, 25 ml dye. It is for 500 assays in a 384-well plate format.

1.4.3 DNA ligase assays

NAD^+-dependent DNA ligases are present in bacteria, some entomopox viruses and mimi virus. Since NAD^+-dependent DNA ligases are essential for bacterial growth, they are valuable targets for identifying novel antibacterial agents. The NAD^+-dependent DNA Ligase Assay Kit is to measure the DNA ligase product in which the diphosphodiester bond is formed at the single stand break of a duplex DNA substrate. The ligase reaction is monitored by the fluorescence intensity at 535 nm. The assay is in 96-well plate format and can be used for screening inhibitors of DNA ligases from gram-positive (such as *S. pneumoniae*) and gram-negative (such as *E. coli*) bacteria.



Assay conditions: 40 µl of reaction volume with 1 nM of NAD-dependent DNA ligase from *S. pneumoniae*, 10 µM NAD, 200 nM substrate DNA in the assay buffer. The EDTA concentration in the control reaction is 50 mM. The assay reaction is at room temperature for 15 min using a 96-well plate.

NAD^+-dependent DNA ligase assay kit (enzyme not included) (Catalog No. NLA100K)

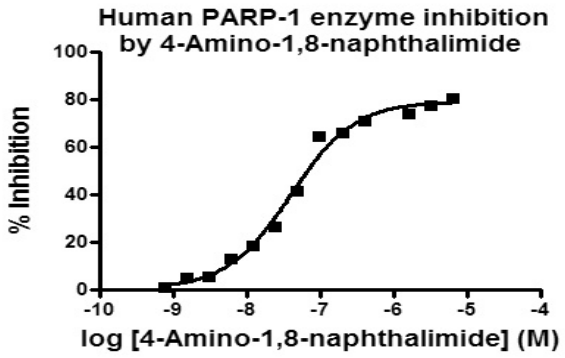
Kit components: 800 µl 10 x Buffer, 44 µl 100x DNA, 220 ul 10 x dye, 21 ml Reagent T, 8 µl 1000 x NAD+. It is for 100 assays in a 96-well plate format.

***E. coli* NAD^+-dependent DNA ligase assay kit Plus (*E. coli* ligase included) (Catalog No. NLA100KE)**

Kit components: 800 µl 10 x Buffer, 44 µl 100 x DNA, 220 µl 10 x dye, 21 ml Reagent T, 8 µl 1000 x NAD^+, 10 ul 500 x *E. coli* ligase. It is for 100 assays in a 96-well plate format.

1.5 Human Parp-1 assay

Poly (ADP-ribose) polymerase-1 (PARP-1) responses to DNA damage and synthesizes poly ADP-ribose (PAR) that is transferred to itself and a variety of acceptor proteins. Excessive activation of PARP-1 not only depletes NAD^+ but also generates toxic PAR that lead to cell death. PARP-1 is an anti-cancer drug target. It is also a potential drug target for other diseases such as stroke, ischemia and reperfusion where high-level DNA damage occurs. The human PARP-1 assay is based on measurement of its product PAR (poly ADP-ribose) that binds to a fluorescent dye and enhances its fluorescent signal.



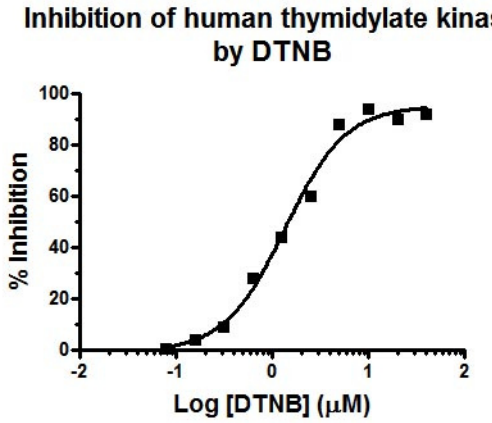
The **Human Poly (ADP-ribose) Polymerase-1 Assay Kit (catalog number PAR100K)** includes 800 µl of 10 x the assay buffer, 55 µl of 100x NAD^+, 55 µl of 100x DNA and 220 µl of 100 x fluorescence dye for 100 assays of human PARP-1 in a 96-well plate format. The kit does not include Human PARP-1.

The **Human Poly (ADP-ribose) Polymerase-1 Assay Kit Plus (catalog number PAR100KE)** includes 800 µl of 10 x the assay buffer, 55 µl of 200x NAD^+, 55 µl of 100x DNA, 17 µl of 300 x Human PARP-1 and 220 µl of 100x fluorescence dye for 100 assays of human PARP-1 in a 96-well plate format.

1.6 Assays for kinases

1.6.1 Human Thymidylate Kinase Assays

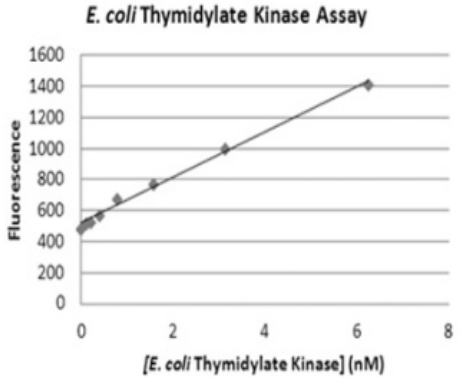
Thymidylate kinase is an important enzyme in the dTTP synthesis pathway for DNA synthesis. The thymidylate kinase activity is higher in the tumor than in the corresponding normal human tissue. The function of this enzyme is to catalyze the phosphorylation of dTMP using ATP to form dTDP and ADP. The thymidylate kinase assay is based on measurement of ADP generated from the kinase reaction. The assay is fluorescence-based and can be carried out using regular black 96-well or 384-well plates or micro-cuvettes.

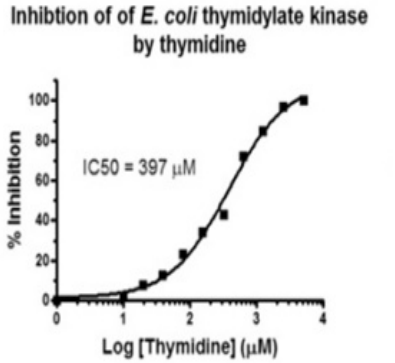


The **Human Thymidylate Kinase Assay Kit Plus-500** (Catalog No. HTMK500KE) includes 1700 µl of 10 x reaction buffer, 8 µl of 2500 x human thymidylate kinase (125 mM), 170 µl of 100 x ATP (5 mM), 170 µl of 100 x dTMP (40 mM), 170 µl of 100 x MUK Reagent A, 170 µl of 100 x MUK Reagent B and 1700 µl of 10 x fluorescence dye. The kit reagents are sufficient for 500 thymidylate kinase assays using a standard black 384-well plate (Matrix 4318).

1.6.2 Bacterial thymidylate kinase

Thymidylate kinase is an important enzyme in the dTTP synthesis pathway for DNA synthesis. The function of this enzyme is to catalyze the phosphorylation of dTMP using ATP to form dTDP and ADP. Thymidylate kinase is an attractive antibacterial target. The thymidylate kinase assay is based on measurement of ADP generated from the kinase reaction. The assay is fluorescence-based and can be carried out using regular black 96-well or 384-well plates or micro-cuvettes.





***E. coli* Thymidylate Kinase Assay Kit Plus (enzyme included) (Catalog No. TMK100KE)**

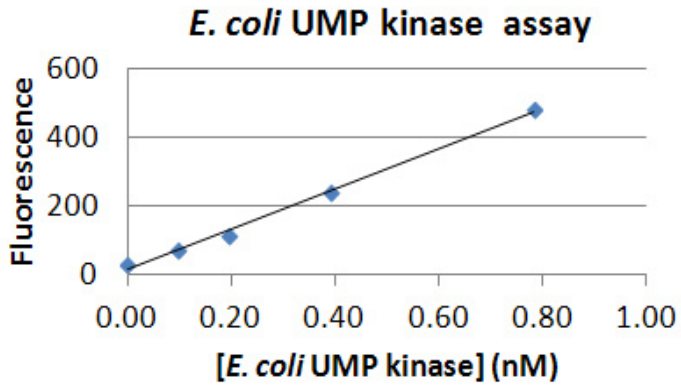
Kit components: 600 µl 10 x Buffer, 35 µl 100x ATP, 35 µl 100 x dTMP, 5 µl 1000x *E.coli* TMK, 35 µl 100x MUK reagent A, 35 µl 100x MUK reagent B, 350 µl 10 x fluorescence dye.

***E. coli* Thymidylate Kinase Assay Kit Plus-500 (enzyme included) (Catalog No. TMK500KE)**

Kit components: 2 ml 10 x Buffer, 170 µl 100 x ATP, 170 µl 100x dTMP, 17 µl 1000 x *E.coli* TMK, 170 µl 100 x MUK reagent A, 170 µl 100x MUK reagent B, 1700 µl 10 x fluorescence dye.

1.6.3 UMP kinase assays

UMP kinase or uridylate kinase is essential for biosynthesis of nucleic acid. It catalyzes the ATP-dependent phosphorylation of UMP into UDP. UMP kinase is an attractive antibacterial target. The UMP kinase assay is based on measurement of ADP generated from the kinase reaction. The assay is fluorescence-based and can be carried out using regular black 96-well or 384-well plates or micro-cuvettes.



***E. coli* UMP Kinase Assay Kit Plus (Catalog No. UMK100KE)**

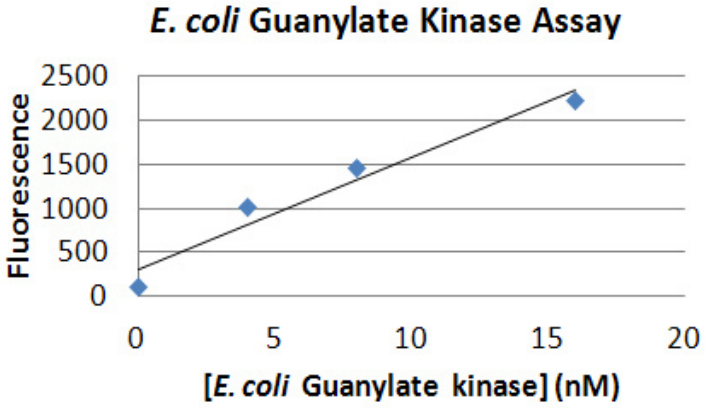
Kit components: 600 µl 10 x Buffer, 35 µl 100x ATP, 35 µl 100 x UMP, 5 µl 1000 x *E.coli* UMK, 35 µl 100 x MUK reagent A, 35 µl 100x MUK reagent B, 350 µl 10 x fluorescence dye.

***E. coli* UMP Kinase Assay Kit Plus-500 (Catalog No. UMK500KE)**

Kit components: 2 ml 10 x Buffer, 170 µl 100x ATP, 170 µl 100x UMP, 17 µl 1000 x *E.coli* UMK, 170 µl 100x MUK reagent A, 170 µl 100 x MUK reagent B, 1700 µl 10 x fluorescence dye.

1.6.4 Guanylate kinase assays

Guanylate kinase is essential for recycling GMP and cGMP. It catalyzes the ATP-dependent phosphorylation of GMP into GDP. Guanylate kinase is an attractive antibacterial target. The guanylate kinase assay is based on measurement of ADP generated from the kinase reaction. The assay is fluorescence-based and can be carried out using regular black 96-well or 384-well plates or cuvettes.



***E. coli* Guanylate Kinase Assay Kit Plus (Catalog No. GMK100KE)**

Kit components: 600 µl 10 x Buffer, 35 µl 100x ATP, 35 µl 100 x GMP, 5 µl 1000 x *E.coli* GMK, 35 µl 100 x MUK reagent A, 35µl 100x MUK reagent B, 350 µl 10 x fluorescence dye.

***E. coli* Guanylate Kinase Assay Kit Plus-500 (Catalog No. GMK500KE)**

Kit components: 2 ml 10 x Buffer, 170 µl 100x ATP, 170 µl 100x GMP, 17 µl 1000 x *E.coli* GMK, 170 µl 100 x MUK reagent A, 170 µl 100x MUK reagent B, 1700 µl 10 x fluorescence dye.

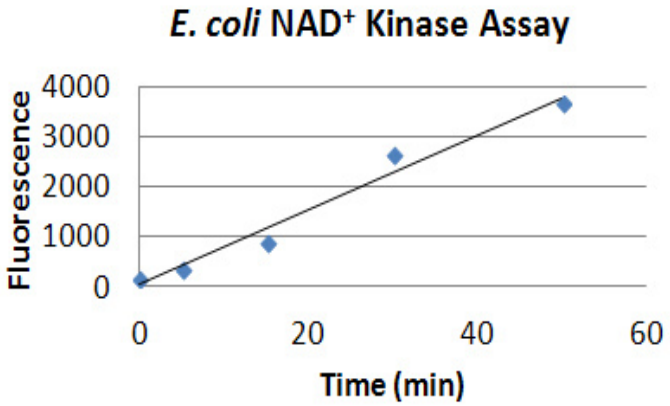
***S. pneumoniae* Guanylate Kinase Assay Kit Plus-500 (Catalog No. GMK500KN)**

Kit components: 2 ml 10 x Buffer, 170 µl 100x ATP, 170 µl 100x GMP, 17 µl 1000x *S. pneumoniae* GMK, 170 µl 100x MUK reagent A, 170 µl 100x MUK reagent B, 1700 µl 10 x fluorescence dye.

1.6.5 NAD^+ kinase assays

NAD^+ kinase converts NAD^+ into $NADP^+$ by phosphorylation in the presence of ATP. $NADP^+$ plays key roles in energy transduction and various biochemical process including DNA repair, protein modification and cell signaling. NAD^+ kinase is an attractive antibacterial target. The

NAD^+ kinase assay is based on measurement of ADP generated from the kinase reaction. The assay is fluorescence-based and can be carried out using regular black 96-well or 384-well plates or micro-cuvettes.



***E. coli* NAD$^+$ Kinase Assay Kit Plus (Catalog No. NAK100KE)**

Kit components: 600 µl 10 x Buffer, 35 µl 100x ATP, 35 µl 100 x NAD^+, 5 µl 1000 x *E.coli* NADK, 35 µl 100 x MUK reagent A, 35 µl 100x MUK reagent B, 350 µl 10 x fluorescence dye.

***E. coli* NAD$^+$ Kinase Assay Kit Plus-500 (Catalog No. NAK500KE)**

Kit components: 2 ml 10 x Buffer, 170 µl 100x ATP, 170 µl 100x NAD, 17 µl 1000 x *E.coli* NADK, 170 µl 100x MUK reagent A, 170 µl 100x MUK reagent B, 1700 µl 10 x fluorescence dye.

1.6.6 Universal Kinase Assay

The MicroMolar Universal Kinase Assay Kit is based on measurement of ADP generated from the kinase reaction. It is used to measure the activities of purified kinases producing ADP at concentrations ranging from 0.1 µM to 10 µM. The assay is fluorescence-based and can be carried out using regular black or white 96-well or 384-well plates or micro-cuvettes. It is important to avoid using unnecessarily high ATP concentrations in the kinase assays for the following reasons. One is to allow the assay to detect ATP-competitive inhibitors. The other is to avoid a high ADP background from ADP contamination in the ATP samples. It is recommended to use ATP concentrations at 20 µM (micromolar).

MicroMolar Universal Kinase Assay Kit (enzyme not included) (Catalog No. MUK100K)

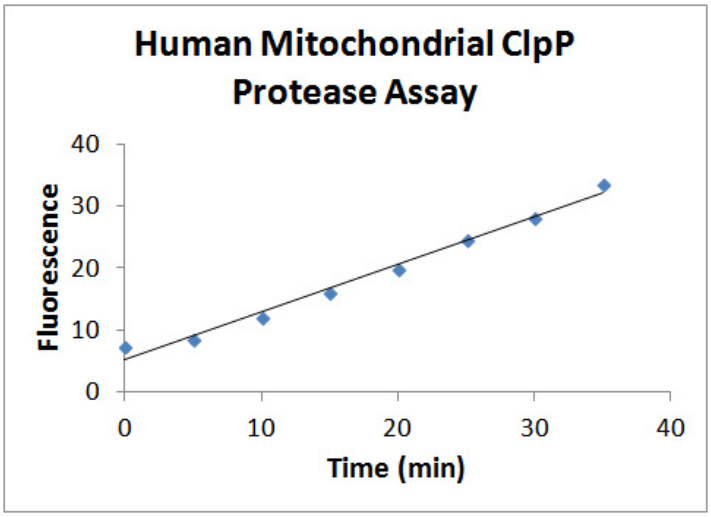
Kit components: 600 µl 10 x MUK Buffer, 35 µl 10 mM ATP, 35 µl 100x MUK reagent A, 35 µl MUK reagent B, 350 µl 10 x fluorescence dye, 30 µl 1 mM ADP.

MicroMolar Universal Kinase Assay Kit-1000 (enzyme not included) (Catalog No. MUK1000K)
Kit components: 4 ml 10 x MUK Buffer, 350 µl 10 mM ATP, 310 µl 100x MUK reagent A, 310 µl MUK reagent B, 3.5 ml 10 x fluorescence dye, 200 µl 1 mM ADP.

1.7 Protease assays

1.7.1 Human ClpP

Human mitochondrial protease ClpP has been identified as a drug target for human acute myeloid leukemia (AML). The high throughput assay for human ClpP protease is based on cleavage of a labeled peptide substrate that generates fluorescence at 460 nm (excitation at 380 nm). The assay can be performed in a 384-well or 96-well plate format for tests of Human ClpP protease activities and throughput screening of inhibitors for cancer therapy.



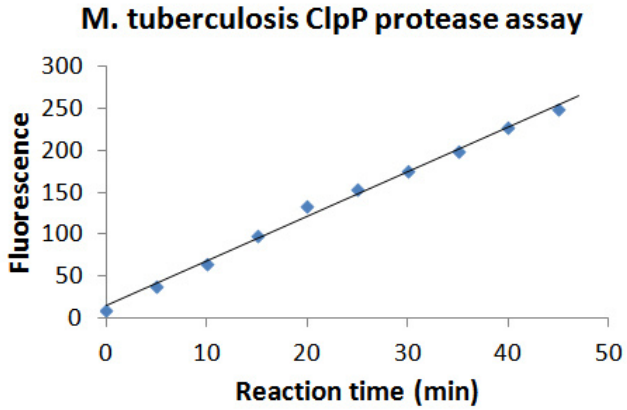
The **Human Mitochondrial ClpP Protease Assay Kit(Catalog No. HMP100K)** includes 3000 µl of Assay Buffer and 310 µl of 10 x Substrate and 3500 µl of Stop solution. It is for 100 assays in 384-well plate format. All assay reagents except the enzyme are included.

The **Human Mitochondrial ClpP Protease Assay Kit Plus (Catalog No. HMP100KE)** includes 3000 µl of Assay Buffer, 310 µl of 10 x Substrate, 3500 µl of Stop solution and 31 µl of 100 x

human ClpP protease. It is for 100 assays in 384-well plate format. All assay reagents are included.

1.7.2 Tuberculosis ClpP

The disease of tuberculosis (TB) is a serious threat to global public health. It is estimated that infection of M. tuberculosis (Mtb) causes more than 1 million deaths annually. The protein degradation enzyme ClpP protease of Mtb is a proven drug target against Mtb. ProFoldin's high throughput assay for Mtb ClpP protease is based on cleavage of a labeled peptide substrate that generates fluorescence at 460 nm (excitation at 380 nm). The assay can be performed in a 384-well or 96-well plate format for tests of Mtb ClpP protease activities and throughput screening of inhibitors against M. tuberculosis.



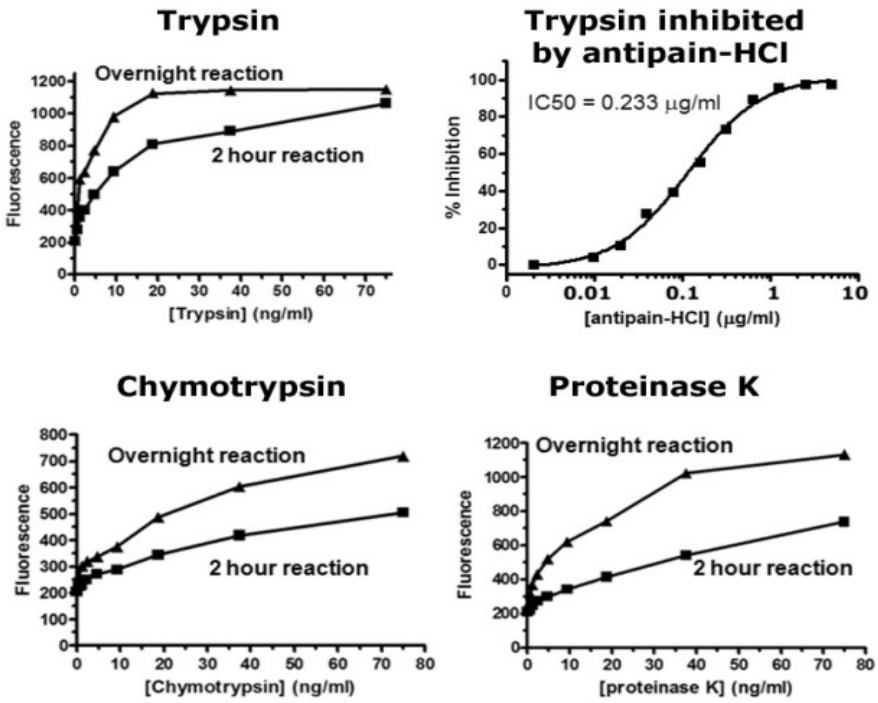
The **M. Tuberculosis ClpP Protease Assay Kit (Catalog No. TBP100K)** includes 3100 µl of Assay Buffer and 310 µl of 10 x Substrate and 3500 µl of Stop solution. It is for 100 assays in 384-well plate format. All assay reagents except the enzyme are included.

The **M. Tuberculosis ClpP Protease Assay Kit Plus (Catalog No. TBP100KE)** includes 3000 µl of Assay Buffer, 310 µl of 10 x Substrate, 3500 µl of Stop solution and 31 µl of 100 x Mtb ClpP1P2 protease. It is for 100 assays in 384-well plate format. All assay reagents are included.

1.7.3 Other protease assays

Protease contamination even in a trace amount can degrade proteins or peptides during purification, storage or crystallization. Protein or peptide drugs should be protease-free. Proteases are also important drug targets. The Ultra-sensitive High-throughput Protease Assay

Kit is useful to detect protease contamination of various protein preparations and to screen protease inhibitors in a 96-well or 384-weel plate assay format. The assay is based on the principle that digestion of the fluorescence-labeled protein substrate results in dramatic increase of the fluorescence signal. The sensitivity of the assay of trypsin, chymotrypsin and proteinase K is below 5 ng/ml or 0.2 ng per assay well for the standard 384-well plate assay.



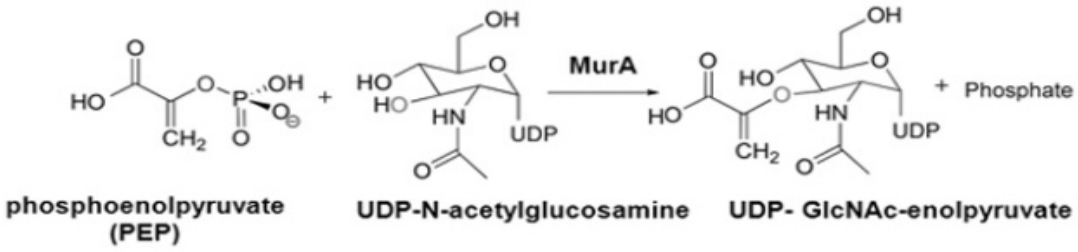
Ultra-Sensitive High-Throughput Protease Assay (Catalog No. UPA1000)
Kit components: 50 μl 1000x substrate, 5 ml 10 x Buffer.

1.8 Assays for bacterial cell wall synthesis enzymes

1.8.1 MurA assays

MurA or UDP-N-acetylglucosamine enolpyruvyl transferase catalyzes the first committed step in peptidoglycan biosynthesis in bacteria. It is an essential enzyme and attractive target for anti-bacterial drug discovery. MurA transfers enolpyruvate from phosphoenolpyruvate (PEP) to uridine diphospho-*N*-acetylglucosamine (UNAG) generating enolpyruvyl-UDPN-acetylglucosamine (EP-UNAG) and inorganic phosphate. The **MurA Assay** is based on measurement of the inorganic phosphate generated from the MurA reaction. The inorganic

phosphate is detected by light absorbance at 650 nm. The assay reactions and detection can be performed by using 384-well or 96-well assay plates. Alternatively, the assay reaction can be carried out in Eppendorf tubes and the signal is measured using a cuvette. The high throughput assay can be used for screening inhibitors of *E.coli* MurA in drug discovery research. It may also be used for characterization of *E.coli* MurA.



***E. coli* MurA Assay Kit Plus-100 (Catalog No. MURA100KE)**

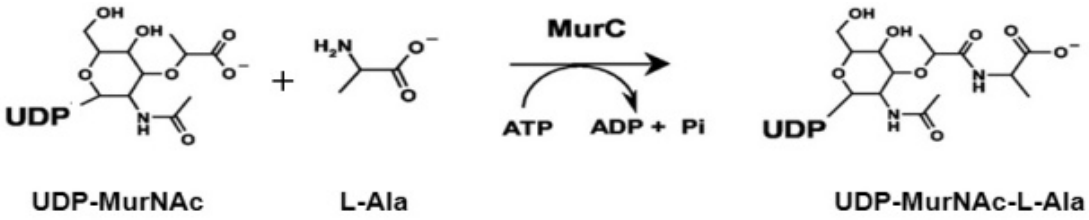
Kit components: 400 µl 10 x Buffer, 35 µl 100 x UGN, 35 µl 100 x PEP, 35 µl 100 x *E. coli* MurA, 5 ml Dye MPA3000.

***E. coli* MurA Assay Kit Plus-500 (Catalog No. MURA500KE)**

Kit components: 2 ml10 x Buffer, 170 µl 100 x UGN, 170 µl 100 x PEP, 170 µl 100 x *E. coli* MurA, 25 ml Dye MPA3000.

1.8.2 MurC assays

MurC or UDP-N-acetylmuramic acid:L-alanine ligase is the first of four paralogous amino acid-adding enzymes in the pathway of peptidoglycan biosynthesis in bacteria. It is an essential enzyme and attractive target for anti-bacterial drug discovery. MurC catalyzes the addition of L-alanine onto the nucleotide precursor UDP-MurNAc generating UDP-MurNAc-L-Ala. The ligation reaction is coupled to the hydrolysis of ATP forming ADP and inorganic phosphate. The **MurC Assay** is based on measurement of the inorganic phosphate generated from the MurC reaction. The inorganic phosphate is detected by light absorbance at 650 nm. The assay reactions and detection can be performed by using 384-well or 96-well assay plates. Alternatively, the assay reaction can be carried out in Eppendorf tubes and the signal is measured using a cuvette if a plate reader is not available. The high throughput assay can be used for screening inhibitors of MurC in drug discovery research. It may also be used for characterization of MurC.



***E. coli* MurC Assay Kit Plus-100 (Catalog No. MURC100KE)**

Kit components: 400 µl 10 x Buffer, 35 µl 100 x UDP-MurAc, 35 µl 100 x L-Ala, 35 µl 100x ATP, 35 µl 100x *E. coli* MurC, 5 ml Dye MPA3000.

***E. coli* MurC Assay Kit Plus-500 (Catalog No. MURC500KE)**

Kit components: 2 ml 10 x Buffer, 170 µl 100 x UDP-MurAc, 170 µl 100 x L-Ala, 170 µl 100x ATP, 170 µl 100 x *E. coli* MurC, 25 ml Dye MPA3000.

***S. pneumoniae* MurC Assay Kit Plus-100 (Catalog No. MURC100KN)**

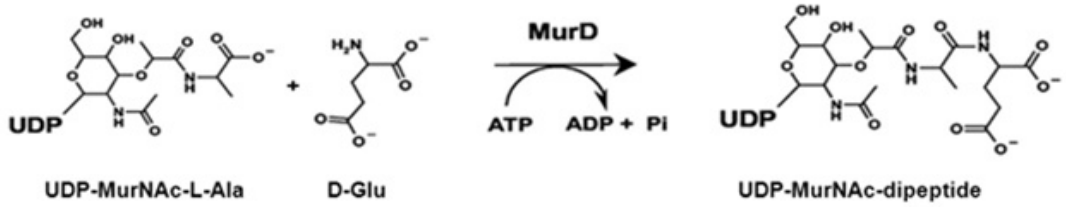
Kit components: 400 µl 10 x Buffer, 35 µl 100 x UDP-MurAc, 35 µl 100 x L-Ala, 35 µl 100x ATP, 35 µl 100 x *S. pneumoniae* MurC, 5 ml Dye MPA3000.

***S. pneumoniae* MurC Assay Kit Plus-500 (Catalog No. MURC500KN)**

Kit components: 2 ml 10 x Buffer, 170 µl 100 x UDP-MurAc, 170 µl 100 x L-Ala, 170 µl 100x ATP, 170 µl 100 x *S. pneumoniae* MurC, 25 ml Dye MPA3000.

1.8.3 MurD assays

MurD is a D-Glutamic acid-adding enzyme in the pathway for bacterial cell-wall peptidoglycan synthesis. It is an essential enzyme and attractive target for anti-bacterial drug discovery. MurD catalyses the addition of D-glutamic acid to UDP-MurNAc-L-Ala, generating UDP-MurNAc-dipeptide. The ligation reaction uses ATP hydrolysis as an energy source forming ADP and inorganic phosphate. The **MurD Assay** is based on measurement of the inorganic phosphate generated from the MurD reaction. The inorganic phosphate is detected by light absorbance at 650 nm. The assay reactions and detection can be performed by using 384-well or 96-well assay plates. Alternatively, the assay reaction can be carried out in Eppendorf tubes and the signal is measured using a cuvette. The high throughput assay can be used for screening inhibitors of MurD in drug discovery research. It may also be used for characterization of MurD.



***E. coli* MurD Assay Kit Plus-100 (Catalog No. MURD100KE)**

Kit components: 400 µl 10 x Buffer, 35 µl 100 x UMA, 35 µl 100 x D-Glu, 35 µl 100 x ATP, 35 µl 100 x *E. coli* MurD, 5 ml Dye MPA3000.

***E. coli* MurD Assay Kit Plus-500 (Catalog No. MURD500KE)**

Kit components: 2 ml 10 x Buffer, 170 µl 100 x UMA, 170 µl 100 x D-Glu, 170 µl 100 x ATP, 170 µl 100 x *E. coli* MurD, 25 ml Dye MPA3000.

***P. aeruginosa* MurD Assay Kit Plus-100 (Catalog No. MURD100KP)**

Kit components: 400 µl 10 x Buffer, 35 µl 100 x UMA, 35 µl 100 x D-Glu, 35 µl 100 x ATP, 35 µl 100 x *P. aeruginosa* MurD, 5 ml Dye MPA3000.

***P. aeruginosa* MurD Assay Kit Plus-500 (Catalog No. MURD500KP)**

Kit components: 2 ml 10 x Buffer, 170 µl 100 x UMA, 170 µl 100 x D-Glu, 170 µl 100 x ATP, 170 µl 100x *P. aeruginosa* MurD, 25 ml Dye MPA3000.

***S. aureus* MurD Assay Kit Plus-100 (Catalog No. MURD100KS)**

Kit components: 400 µl 10 x Buffer, 35 µl 100 x UMA, 35 µl 100 x D-Glu, 35 µl 100 x ATP, 35 µl 100 x *S. aureus* MurD, 5 ml Dye MPA3000.

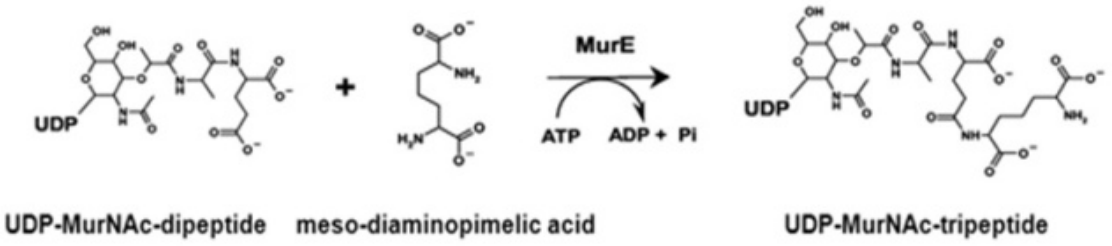
***S. aureus* MurD Assay Kit Plus-500 (Catalog No. MURD500KS)**

Kit components: 2 ml 10 x Buffer, 170 µl 100 x UMA, 170 µl 100 x D-Glu, 170 µl 100x ATP, 170 µl 100 x *S. aureus* MurD, 25 ml Dye MPA3000.

1.8.4 MurE assays

MurE or UDP-MurNAc-tripeptide ligase is the third amino acid-adding enzymes in the pathway of peptidoglycan biosynthesis in bacteria . It is an essential enzyme and attractive target for

anti-bacterial drug discovery. MurE catalyses the addition of lysine or meso-diaminopimelic acid (DAP) into the MurD product UDP-MurNAc-dipeptide in bacteria generating the UDP-MurNAc-tripeptide. The ligation reaction is coupled to the hydrolysis of ATP forming ADP and inorganic phosphate. The **MurE Assay** is based on measurement of the inorganic phosphate generated from the MurE reaction. The inorganic phosphate is detected by light absorbance at 650 nm. The assay reactions and detection can be performed by using 384-well or 96-well assay plates. Alternatively, the assay reaction can be carried out in Eppendorf tubes and the signal is measured using a cuvette. The high throughput assay can be used for screening inhibitors of MurE in drug discovery research. It may also be used for characterization of MurE.



***E. coli* MurE Assay Kit Plus-100 (Catalog No. MURE100KE)**

Kit components: 400 µl 10 x Buffer, 35 µl 100 x DAP, 35 µl 100 x UMAG, 35 µl 100x ATP, 35 µl 100 x *E. coli* MurE, 5 ml Dye MPA3000.

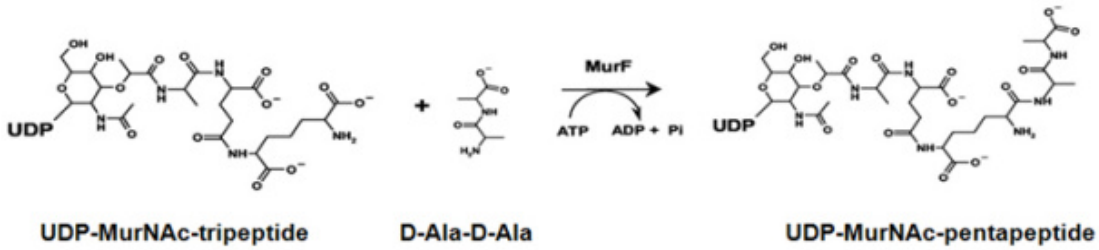
***E. coli* MurE Assay Kit Plus-500 (Catalog No. MURE500KE)**

Kit components: 2 ml 10 x Buffer, 170 µl 100 x DAP, 170 µl 100 x UMAG, 170 µl 100x ATP, 170 µl 100 x *E. coli* MurE, 25 ml Dye MPA3000.

1.8.5 MurF assays

MurF is the enzyme that catalyzes the last step in synthesis of UDP-MurNAc-pentapeptide in the pathway of peptidoglycan biosynthesis in bacteria. It is an essential enzyme and attractive target for anti-bacterial drug discovery. MurF adds a dipeptide D-Ala-D-Ala onto the MurE product UDP-MurNAc-tripeptide. The ligation reaction is coupled to the hydrolysis of ATP forming ADP and inorganic phosphate. The **MurF Assay** is based on measurement of the inorganic phosphate generated from the MurF reaction. The inorganic phosphate is detected by light absorbance at 650 nm. The assay reactions and detection can be performed by using 384-well or 96-well assay plates. Alternatively, the assay reaction can be carried out in

Eppendorf tubes and the signal is measured using a cuvette. The high throughput assay can be used for screening inhibitors of MurF in drug discovery research. It may also be used for characterization of MurF.



***E. coli* MurF Assay Kit Plus-100 (Catalog No. MURF100KE)**

Kit components: 400 µl 10 x Buffer, 35 µl 100 x UMAG-DAP, 35 µl 100 x DAA, 35 µl 100x ATP, 35 µl 100x *E. coli* MurF, 5 ml Dye MPA3000.

***E. coli* MurF Assay Kit Plus-500 (Catalog No. MURF500KE)**

Kit components: 2 ml 10 x Buffer, 170 µl 100 x UMAG-DAP, 170 µl 100 x DAA, 170 µl 100x ATP, 170 µl 100x *E. coli* MurF, 25 ml Dye MPA3000.

***P. aeruginosa* MurF Assay Kit Plus-100 (Catalog No. MURF100KP)**

Kit components: 400 µl 10 x Buffer, 35 µl 100 x UMAG-DAP, 35 µl 100 x DAA, 35 µl 100x ATP, 35 µl 100x *P. aeruginosa* MurF, 5 ml Dye MPA3000.

***P. aeruginosa* MurF Assay Kit Plus-500 (Catalog No. MURF500KP)**

Kit components: 2 ml 10 x Buffer, 170 µl 100 x UMAG-DAP, 170 µl 100 x DAA, 170 µl 100x ATP, 170 µl 100x P. aeruginosa MurF, 25 ml Dye MPA3000.

***S. aureus* MurF Assay Kit Plus-100 (Catalog No. MURF100KS)**

Kit components: 400 µl 10 x Buffer, 35 µl 100 x UMAG-DAP, 35 µl 100 x DAA, 35 µl 100x ATP, 35 µl 100x *S. aureus* MurF, 5 ml Dye MPA3000.

***S. aureus* MurF Assay Kit Plus-500 (Catalog No. MURF500KS)**

Kit components: 2 ml 10 x Buffer, 170 µl 100 x UMAG-DAP, 170 µl 100 x DAA, 170 µl 100x ATP, 170 µl 100x *S. aureus* MurF, 25 ml Dye MPA3000.

***S. pneumoniae* MurF Assay Kit Plus-100 (Catalog No. MURF100KN)**

Kit components: 400 µl 10 x Buffer, 35 µl 100 x UMAG-DAP, 35 µl 100 x DAA, 35 µl 100x ATP, 35 µl 100x *S. pneumoniae* MurF, 5 ml Dye MPA3000.

***S. pneumoniae* MurF Assay Kit Plus-500 (Catalog No. MURF500KN)**

Kit components: 2 ml 10 x Buffer, 170 µl 100 x UMAG-DAP, 170 µl 100 x DAA, 170 µl 100x ATP, 170 µl 100x *S. pneumoniae* MurF, 25 ml Dye MPA3000.

1.8.6 D-alanine ligase Assays

D-Alanine-D-Alanine is one of the building blocks in peptidoglycan biosynthesis in bacteria. This dipeptide is generated by ligation between two D-Alanine molecules catalyzed by D-Alanine : D-Alanine ligase. The ligation reaction is coupled to the hydrolysis of ATP forming ADP and inorganic phosphate. The ***E. coli* D-Alanine :D-Alanine Ligase Assay** is based on measurement of the inorganic phosphate generated from the D-Alanine : D-Alanine ligation reaction. The inorganic phosphate is detected by light absorbance at 650 nm. The assay reactions and detection can be performed by using 384-well or 96-well assay plates. Alternatively, the assay reaction can be carried out in Eppendorf tubes and the signal is measured using a cuvette. The high throughput assay can be used for screening inhibitors of *E.coli* D-Alanine : D-Alanine ligase in drug discovery research. It may also be used for characterization of *E.coli* D-Alanine : D-Alanine ligase.

***E. coli* D-alanine ligase Assay Kit Plus-100 (Catalog No. DDA100KE)**

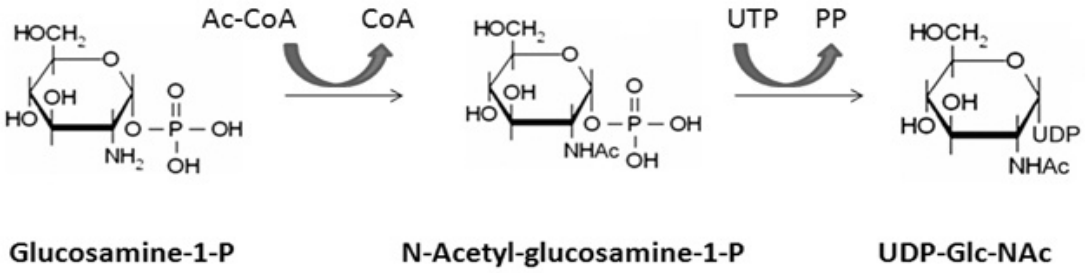
Kit components: 400 µl 10 x Buffer, 35 µl 100 x DAA, 35 µl 100 x ATP, 35 µl 100x *E. coli* Ddl, 5 ml Dye MPA3000.

***E. coli* D-alanine ligase Assay Kit Plus-500 (Catalog No. DDA500KE)**

Kit components: 2 ml 10 x Buffer, 170 µl 100 x DAA, 170 µl 100 x ATP, 170 µl 100x *E. coli* Ddl, 25 ml Dye MPA3000.

1.8.7 UDP-N-acetylglucosamine pyrophosphorylase (GlmU) assays

UDP-N-acetylglucosamine pyrophosphorylase (GlmU) is a bifunctional enzyme that catalyzes transfer of acetyl and uridyl groups onto glucosamine-1-P to generate UDP-GlcNAc, an essential precursor of peptidoglycans. UDP-GlcNAc is involved in the synthesis of the N-acetylglucosamine polysaccharide adhesin required for biofilm formation in bacteria. The ***E. coli* UDP-N-acetylglucosamine pyrophosphorylase (GlmU) Assay** is based on measurement of the pyrophosphate generated from the GlmU reaction. The pyrophosphate is converted to phosphate by pyrophosphatase and the phosphate is detected by light absorbance at 650 nm. The assay reactions and detection can be performed by using 384-well or 96-well assay plates. Alternatively, the assay reaction can be carried out in Eppendorf tubes and the signal is measured using a cuvette. The high throughput assay can be used for screening inhibitors of *E.coli* GlmU in drug discovery research. It may also be used for characterization of *E.coli* GlmU.



***E. coli* GlmU Assay Kit Plus-100 (Catalog No. GLU100KE)**

Kit components: 400 µl 10 x Buffer, 35 µl 100 x G-1-P, 35 µl 100 x Ac-CoA, 35 µl 100 x ppase, 35 µl 100x *E. coli* GlmU, 5 ml Dye MPA3000.

***E. coli* GlmU Assay Kit Plus-500 (Catalog No. GLU500KE)**

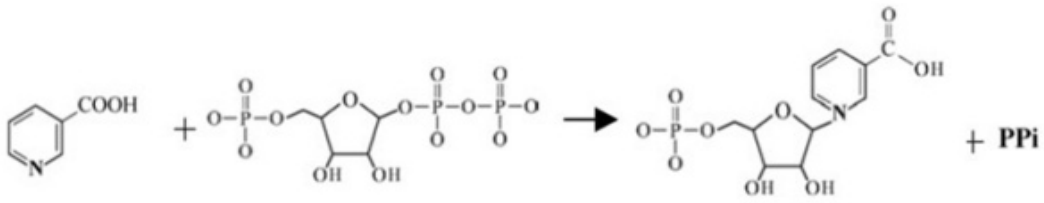
Kit components: 2 ml10 x Buffer, 170 µl 100 x G-1-P, 170 µl 100 x Ac-CoA, 170 µl 100x ppase, 170 µl 100x *E. coli* GlmU, 25 ml Dye MPA3000.

1.9 Assays for other anti-bacterial target enzymes

1.9.1 Nicotinate phosphoribosyltransferase (pncB) assays

Nicotinate phosphoribosyltransferase (NAPRTase or PNCB) provides a pathway of regenerating nicotinic acid adenine dinucleotide (NAD) through synthesizing nicotinic acid mononucleotide (NAMN). The enzyme transfers nicotinate (NA) onto phosphoribosyl pyrophosphate (PRPP)

releasing pyrophosphate. PNCB is drug target for gram (+) bacteria. The **Nicotinate Phosphoribosyltransferase (PNCB) Assay** is based on measurement of the pyrophosphate generated from the enzyme reaction. The pyrophosphate is converted to phosphate by pyrophosphatase and the phosphate is detected by light absorbance at 650 nm. The assay reactions and detection can be performed by using 384-well or 96-well assay plates. Alternatively, the assay reaction can be carried out in Eppendorf tubes and the signal is measured using a cuvette. The high throughput assay can be used for screening inhibitors of PNCB in drug discovery research. It may also be used for characterization of PNCB enzyme.



***S. pneumonia* PncB Assay Kit Plus-100 (Catalog No. PNCB100KN)**

Kit components: 400 µl 10 x Buffer, 35 µl 100 x PRPP, 35 µl 100 x NA, 35 µl 100x ATP, 35 µl 100x Ppase, 35 µl 100x *S. pneumoniae* pncB, 5 ml Dye MPA3000.

***S. pneumonia* PncB Assay Kit Plus-500 (Catalog No. PNCB500KN)**

Kit components: 2 ml 10 x Buffer, 170 µl 100 x PRPP, 170 µl 100 x NA, 170 µl 100x ATP, 170 µl 100x Ppase, 170 µl 100x *S. pneumoniae* pncB, 25 ml Dye MPA3000.

***S. aureus* PncB Assay Kit Plus-100 (Catalog No. PNCB100KS)**

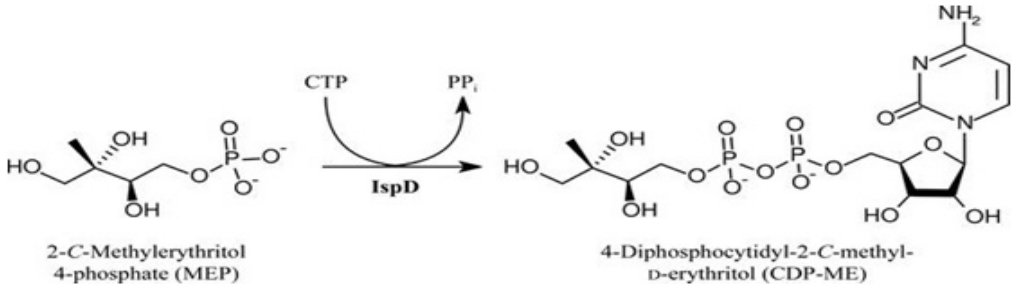
Kit components: 400 µl 10 x Buffer, 35 µl 100 x PRPP, 35 µl 100 x NA, 35 µl 100x ATP, 35 µl 100x Ppase, 35 µl 100x *S.aureus* pncB, 5 ml Dye MPA3000.

***S. aureus* PncB Assay Kit Plus-500 (Catalog No. PNCB500KS)**

Kit components: 2 ml 10 x Buffer, 170 µl 100 x PRPP, 170 µl 100 x NA, 170 µl 100x ATP, 170 µl 100x Ppase, 170 µl 100x *S. aureus* pncB, 25 ml Dye MPA3000.

1.9.2 Methylerythritol phosphate cytidyltransferase (IspD) assays

Methylerythritol phosphate cytidyltransferase (IspD) is one of the enzymes in the non-mevalonate pathway for isoprenoid biosynthesis that present in many pathogenic organisms and plants but absent in mammals. IspD is an attractive target for the development of novel antibiotics and herbicides. This enzyme catalyzes the formation of 4-diphosphocytidyl-2-C-methyl-D-erythritol from CTP and 2-C-methyl-D-erythritol 4-phosphate (MEP). The **Methylerythritol Phosphate Cytidyltransferase (IspD) Assay** is based on measurement of the pyrophosphate generated from the IspD reaction. The pyrophosphate is converted to phosphate by pyrophosphatase and the phosphate is detected by light absorbance at 650 nm. The assay is in a 384-well or 96-well plate format. It can be used for high throughput screening in drug discovery.



***E. coli* IspD Assay Kit Plus-100 (Catalog No. ISPD100KE)**

Kit components: 400 µl 10 x Buffer, 35 µl 100 x MEP, 35 µl 100 x CTP, 35 µl 100x ATP, 35 µl 100x Ppase, 35 µl 100x *E. coli* IspD, 5 ml Dye MPA3000.

***E. coli* IspD Assay Kit Plus-500 (Catalog No. ISPD500KE)**

Kit components: 2 ml10 x Buffer, 170 µl 100 x MEP, 170 µl 100 x CTP, 170 µl 100x ATP, 170 µl 100x Ppase, 170 µl ul 100x *E. coli* IspD, 5 ml Dye MPA3000.

***P. aeruginosa* IspD Assay Kit Plus-100 (Catalog No. ISPD100KP)**

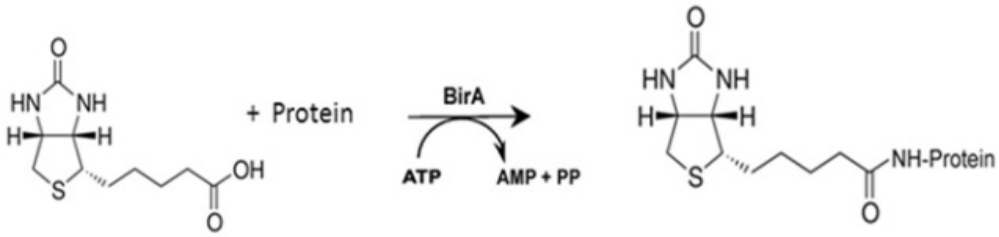
Kit components: 400 ul 10 x Buffer, 35 µl 100 x MEP, 35 µl 100 x CTP, 35 µl 100x ATP, 35 µl 100x Ppase, 35 µl 100x *P. aeruginosa* IspD, 5 ml Dye MPA3000.

***P. aeruginosa* IspD Assay Kit Plus-500 (Catalog No. ISPD500KP)**

Kit components: 2 ml10 x Buffer, 170 µl 100 x MEP, 170 µl 100 x CTP, 170 µl 100x ATP, 170 µl 100x Ppase, 170 µl 100x *P. aeruginosa* IspD, 5 ml Dye MPA3000.

1.9.3 Biotin protein ligase (BirA) assays

Biotin protein ligase (BPL or BirA) is responsible for biotinylation of biotin-dependent proteins. It is an essential enzyme and an attractive target for anti-bacterial drug discovery. The ***E. coli* Biotin Protein Ligase Assay** is based on measurement of the pyrophosphate generated from the BirA reaction using hydroxylamine in the place of protein as a biotin receptor. The pyrophosphate is converted to phosphate by pyrophosphatase and the phosphate is detected by light absorbance at 650 nm. The assay reactions and detection can be performed by using 384-well or 96-well assay plates. Alternatively, the assay reaction can be carried out in Eppendorf tubes and the signal is measured using a cuvette. The high throughput assay can be used for screening inhibitors of *E.coli* BirA in drug discovery research. It may also be used for characterization of *E.coli* BirA.

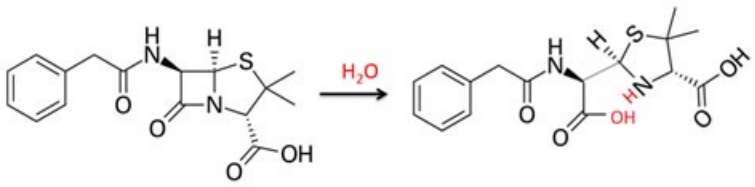


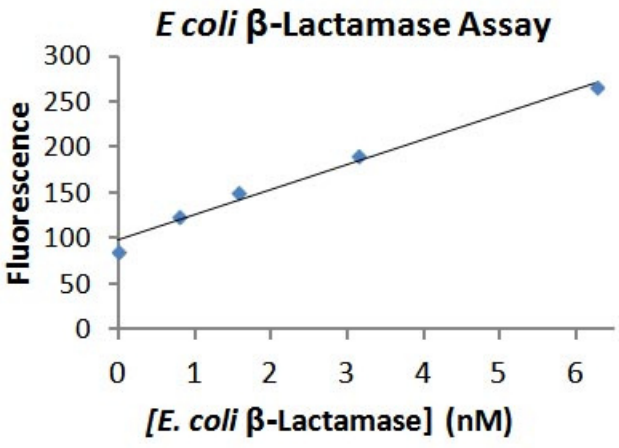
***E. coli* Biotin protein ligase assay kit plus (Catalog No. BPL100KE)**

Kit components: 400 μl 10 x Buffer, 35 μl 100 x biotin, 35 μl 100 x ATP, 35 ul 100 x hydroxylamine hydrochloride, 35 μl 100 x ppase, 35 μl 100 x *E. coli* BirA, 5 ml MPA3000.

1.9.4 Bacterial beta lactamase

Resistance to β-lactam antibiotics such as penicillins, cephalosporins, cephamycins and carbapenems are due to hydrolysis of the four-atom ring known as β-lactam by β-lactamases (penicillinase) produced by Gram-negative bacteria. Inhibition of β-lactamases prevents bacterial degradation of beta-lactam antibiotics. ProFoldin's high throughput assay for β-lactamases is based on binding of the dye with the hydrolyzed β-lactam that generates fluorescence at 535 nm (excitation at 485 nm). The assay can be performed in a 384-well or 96-well plate format for tests of β-lactamase activities and throughput screening of inhibitors against β-lactamases.





The **Beta Lactamase Assay Kit (Catalog No. LAC100K)** includes 550 µl of 10 x Buffer and 110 µl of 50 x penicillin G and 260 µl of 10 x fluorescence dye. It is for 100 assays of β-lactamases in a 96-well plate or 384-well plate format. All assay reagents except the enzyme are included.

The ***E coli*** **Beta Lactamase Assay Kit Plus (Catalog No. LAC100KE)** includes 550 µl of 10 x Buffer and 110 µl of 50 x penicillin G, 260 µl of 10 x fluorescence dye and 55 µl of 100 x *E. coli* β-lactamases. It is for 100 assays of β-lactamases in a 96-well plate or 384-well plate format.

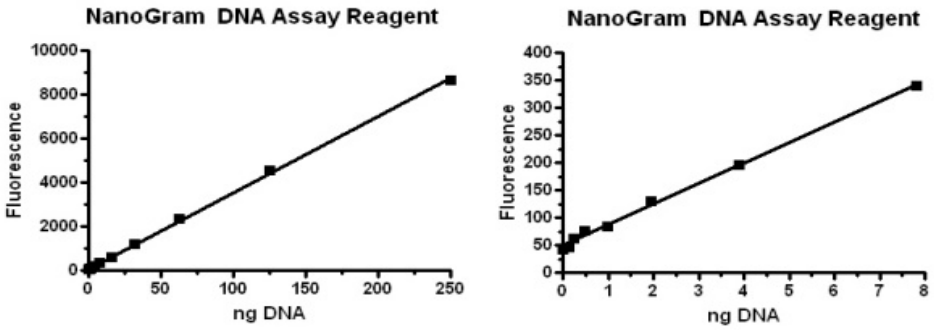
Chapter 2
Concentration assays of various molecules and ions

Concentration measurement is an essential need in all laboratories from life sciences to chemistry in academia and industry. Many of the concentration assays of biomolecules are the foundation of characterization of biological process involving enzymatic reactions or translocation of biomolecules. Others are used for monitoring chemical process, characterization of pharmaceutical or other products or materials from biological or environment sources. ProFoldin provides concentration assays of various biomolecules including DNA, protein, ADP, UDP, GDP, histidine, cysteine, other amino acids, peptides, CoA, lipids and phospholipids. ProFoldin also offers concentration assays of various organic and inorganic molecules, ions and drug molecules including DTT, EDTA, detergent, detergent CMC, SDS, phosphate, polyphosphate, sulfate, chloride, Zn^{++}, Cu^{++}, Co^{++}, Ni^{++}, penicillin drugs, polypeptide drugs, Cisplatin and Oxaliplatin drugs.

2.1 Concentration assays for biomolecules

2.1.1 DNA

The NanoGram DNA Assay Reagent is for measurement of a wide range of DNA or RNA from nanograms to micrograms. The assay is based on the principle that DNA binds to the NanoGram fluorescence dye (Reagent D) and enhances the fluorescence intensity at 535 nm (excitation 485 nm).

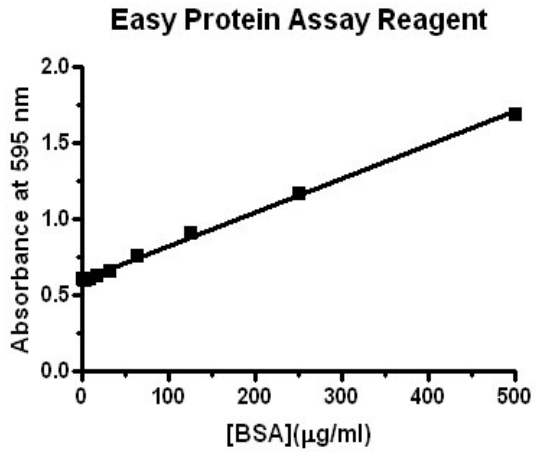


NanoGram DNA Assay Reagent (Catalog No. NDA500)

Kit components: 5 ml 10 x Reagent D.

2.1.2 Protein

The Easy Protein Assay Reagent provides a quick and simple method to measure protein concentrations. The assay is based on protein - Coomassie Blue binding and its light absorbance 595 nm. The assay is quick and easy: simply mix the diluted reagent with 1/10 volume of the protein solution and read the light absorbance at 595 nm. Standard clear 96-well plates or cuvettes can be used for absorbance reading.

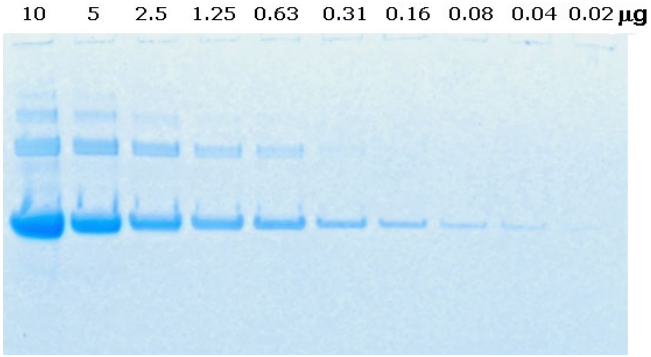


Easy Protein Assay Reagent (Catalog No. EPA001)

Kit components: 100 ml 5 x reagent.

Environment-friendly SDS-PAGE staining

The Easy Protein SDS-PAGE Staining Solution is designed to avoid air pollution in the lab. The staining solution does not contain acetic acid or methanol. After a brief staining, the stained gel is simply destained in water. The gel staining is based on protein – Coomassie Blue binding that gives blue color.

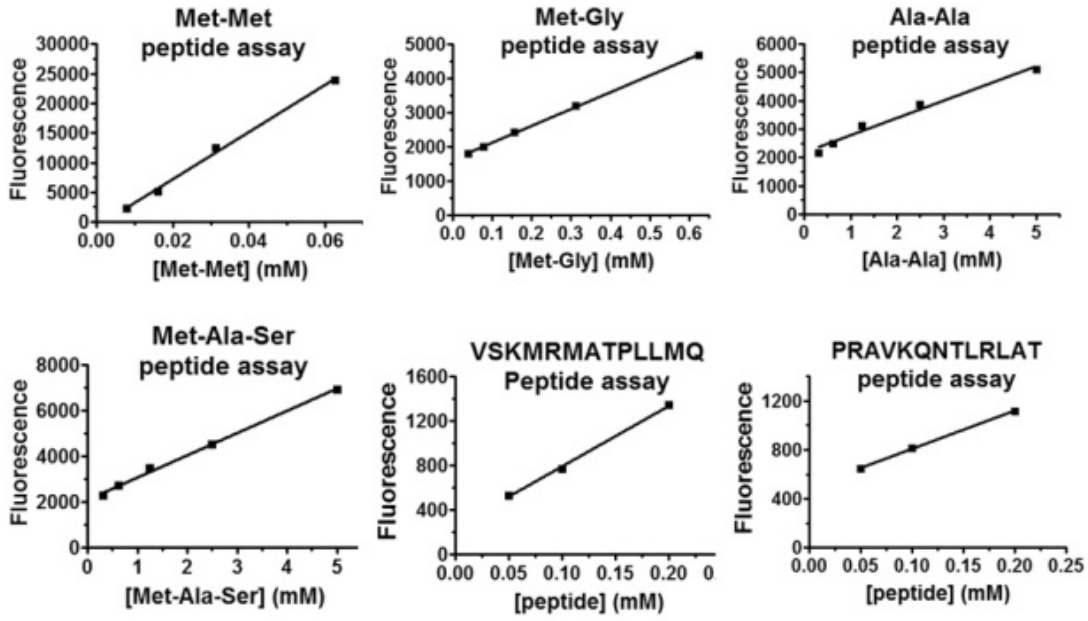


Easy Protein SDS-PAGE staining solution (Catalog No. EPS001)

Kit components: 950 ml staining solution.

2.1.3 Peptide

Short amino acid chains or peptides play key roles in many biological functions. Peptides from antigens are responsible for immune responses. Many biologically secreted and synthetic peptides have been used or being developed as drugs in various therapeutic areas including treatment of infection, cancer, diabetes, and cardiovascular diseases. The MicroMolar Peptide Assay Kit (Catalog No PEP200) is designed for concentration measurement of various peptides. The assay is based on increase of fluorescence at 535 nm of the dye C57 in the presence of peptides. The assay kit can be used for measurements peptide concentrations in synthetic or biochemical reactions, pharmaceutical products and environmental water samples.

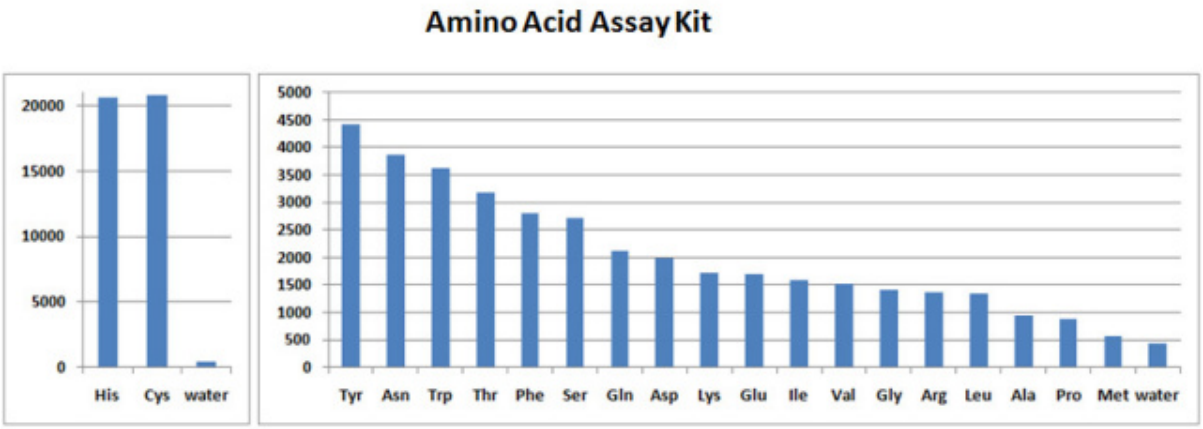


The MicroMolar Peptide Assay Kit (Catalog No PEP200) provides the reagent for measurement of 200 samples using 96-well plates. Cuvettes may also be used for measurements. The assay sensitivity varies from micromolar to millimolar concentrations depending on the nature of peptides. The assay is compatible with HEPES buffer, low concentrations of non-ionic detergent (<0.01%), $MgCl_2$ (< 5 mM), $CaCl_2$ (<5 mM), EDTA (< 1 mM) and phosphate (< 1 mM). It is not compatible with thiol compounds such as DTT.

2.1.4 Amino acids, histidine, cysteine

Amino acid assay

The Amino Acid Assay Kit (Catalog No AAK1000) is for measurement of micromolar to millimolar concentrations of amino acids. The assay sensitivity is 0.01 mM for His and Cys and less than 0.5 mM for most amino acids. The assay is based on the principle that amino acids interacts with dye C53 and enhance the fluorescence intensity at 535 nm (excitation at 485 nm).



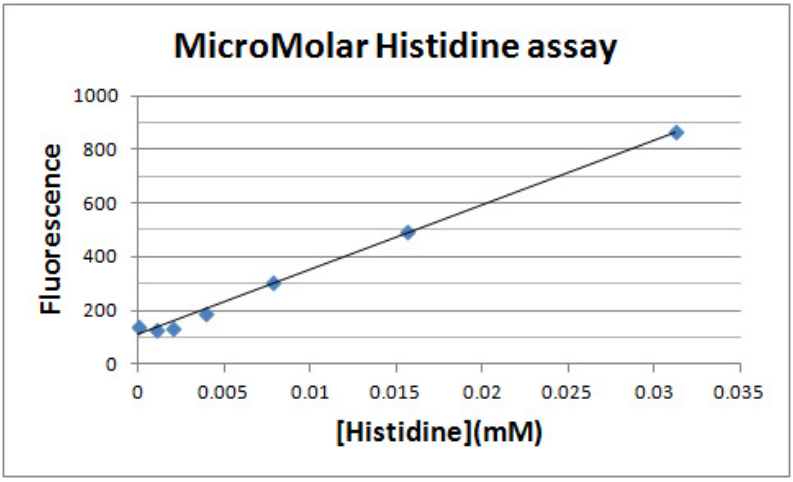
Fluorescence signal at 535 nm (excitation at 485 nm) was measured after mixing 0.1 ml of 0.625 mM amino acid and 0.025 ml of the assay reagent at room temperature for 5 min.

The assay reagent is sufficient for measurement of 1000 samples using 384-well or 96- well plates. It can also be used for measurement of amino acid concentrations using cuvettes. The assay is compatible with HEPES buffer, low concentrations of non-ionic detergent (<0.01%), $MgCl_2$(< 5 mM), $CaCl_2$(<5 mM) and phosphate (< 1 mM). The assay is not compatible with Tris buffer or other primary amine buffers. It is not compatible with DTT or EDTA.

The assay kit (Catalog No AAK1000) includes 1 ml of 10 x dye C53. It is for 1000 assays using 384-well plates or 250 assays using 96-well plates. Cuvettes may also be used for the assays.

Histidine assay

Histidine is an important molecule in biology and pharmaceutical science. Metabolic block of histidine results in increased concentrations of histidine in blood, urine, and cerebrospinal fluid. Disorders of histidine metabolism is related to diseases such as histidinemia and urocanic aciduria. Histidine is a common ingredient of pharmaceutical products. The MicroMolar Histidine Assay Kit (Catalog No HIS200) is designed for measurement of micromolar concentrations of histidine.

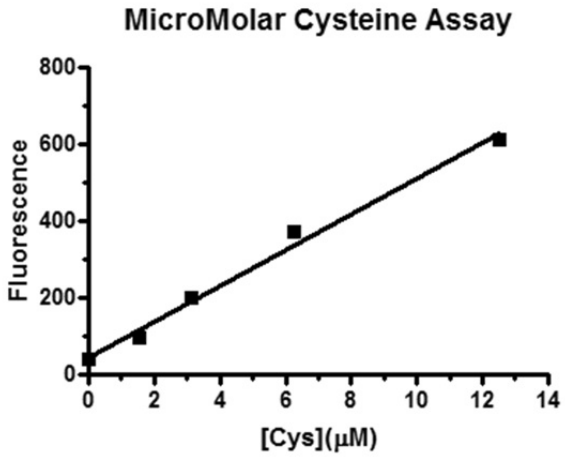


The assay is based on increase of fluorescence at 535 nm of the dye C53 in the presence of histidine. The assay kit can be used for measurements histidine concentrations in biological samples, biochemical reactions, pharmaceutical products and environmental water samples. The assay is compatible with HEPES buffer, low concentrations of non-ionic detergent (<0.01%), $MgCl_2$ (< 5 mM), $CaCl_2$ (<5 mM), EDTA (< 1 mM) and phosphate (< 1 mM). It is not compatible with thiol compounds such as DTT. For measurement of other amino acids, see the information of Amino Acid Assay Kit (Catalog No AAK1000).

The **MicroMolar Histidine Assay Kit (Catalog No HIS200)** provides the reagent for measurement of 200 samples using 96-well plates. Cuvettes may also be used for measurements.

Cysteine assay

Abnormal cysteine levels in vivo are related to cardiovascular disease, Huntington's disease, HIV infection and cancer. Cysteine is a common reducing agent in many in vitro experiments. The MicroMolar Cysteine Assay Kit (Catalog No CYS200) is designed for measurement of micromolar concentrations of cysteine. The assay is based on increase of fluorescence at 535 nm of the dye R53 in the presence of cysteine. The assay kit can be used for measurements cysteine concentrations in biochemical reactions, pharmaceutical products and environmental water samples.



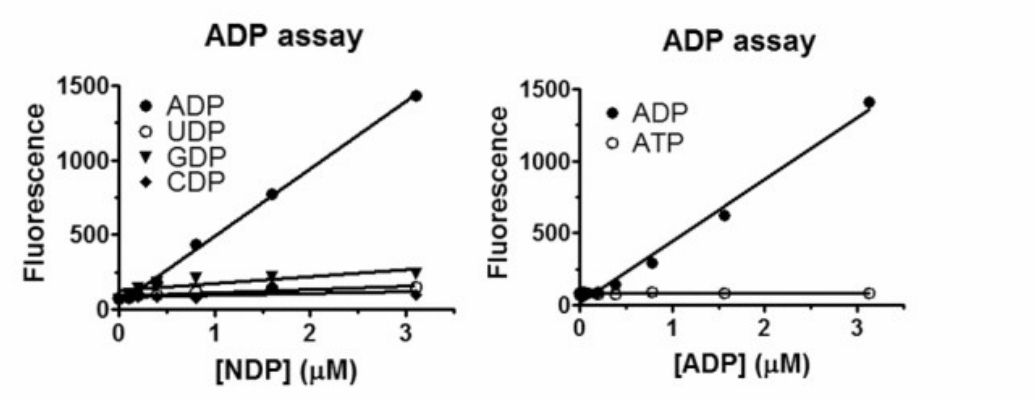
The **MicroMolar Cysteine Assay Kit (Catalog No CYS200)** provides the reagent for measurement of 200 samples using 96-well plates. Cuvettes or 383-well plates may also be used for measurements.

The assay is compatible with HEPES buffer. It is not compatible with other thiol compounds such as DTT. For measurement of DTT, see the information of MicroMolar DTT Assay Kit (Catalog No: DTT200).

2.1.5 NDPs - ADP, UDP, GDP concentrations

ADP

The MicroMolar ADP Assay is for measurement of ADP concentrations in a sub-micromolar to low micromolar range. Other nucleoside diphosphates (UDP, GDP and CDP) showed very little background. Nucleoside triphosphates (ATP, UTP, GTP and CTP) and monophosphates (AMP, UMP, GMP and CMP) are not detected. The ADP assay is based on fluorescence measurement with emission at 535 nm and excitation at 485 nm. It is a high throughput assay using regular black 96-well or 384-well plates. Micro-cuvettes may also be used for detection of the fluorescence signals. The assay can be used to measure contamination of ADP in ATP samples or monitor hydrolysis of ATP. It can also be used for measurement of kinase activities and other enzyme activities that generate ADP molecules. The ADP assay is compatible with common reaction buffers with magnesium. It is not compatible with samples containing high concentrations of DNA or RNA.



MicroMolar ADP Assay kit - 100 assays (Catalog No. MAD100K)

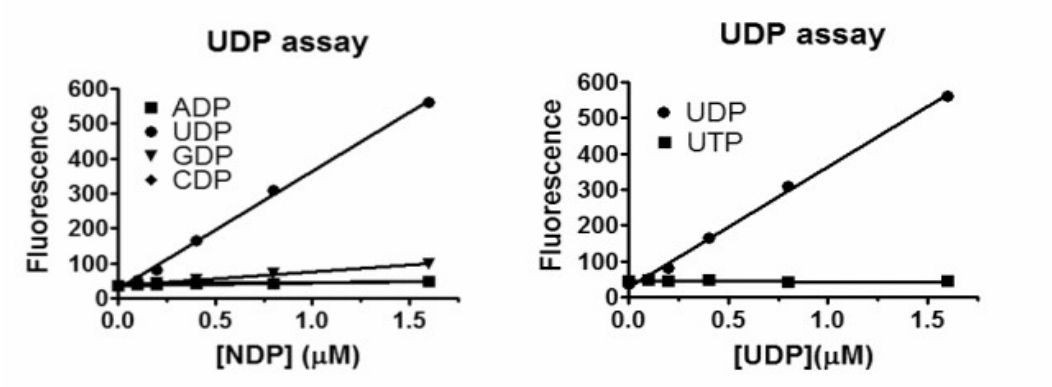
Kit components: 350 μl 10 x Buffer, 33 μl 100x MAD reagent 1, 33 μl 100x Mad reagent 2, 330 μl 10 x fluorescence dye, 30 μl 1 mM ADP.

MicroMolar ADP Assay kit - 500 assays (Catalog No. MAD500K)

Kit components: 1800 μl 10 x Buffer, 165 μl 100x MAD reagent 1, 165 μl 100x Mad reagent 2, 1800 μl 10 x fluorescence dye, 100 μl 1 mM ADP.

UDP

The MicroMolar UDP Assay is for measurement of UDP concentrations in a sub-micromolar to low micromolar range. Other nucleoside diphosphates (ADP, GDP and CDP) showed very little background. Nucleoside triphosphates (ATP, UTP, GTP and CTP) and monophosphates (AMP, UMP, GMP and CMP) are not detected. The UDP assay is based on fluorescence measurement with emission at 535 nm and excitation at 485 nm. It is a high throughput assay using regular black 96-well or 384-well plates. Micro-cuvettes may also be used for detection of the fluorescence signals. The assay can be used to measure contamination of UDP in UTP samples or monitor hydrolysis of UTP. It can also be used for measurement of enzyme activities that generate UDP molecules. The UDP assay is compatible with common reaction buffers with magnesium. It is not compatible with samples containing high concentrations of DNA or RNA.



MicroMolar UDP assay kit - 100 assays (Catalog No. MUD100K)

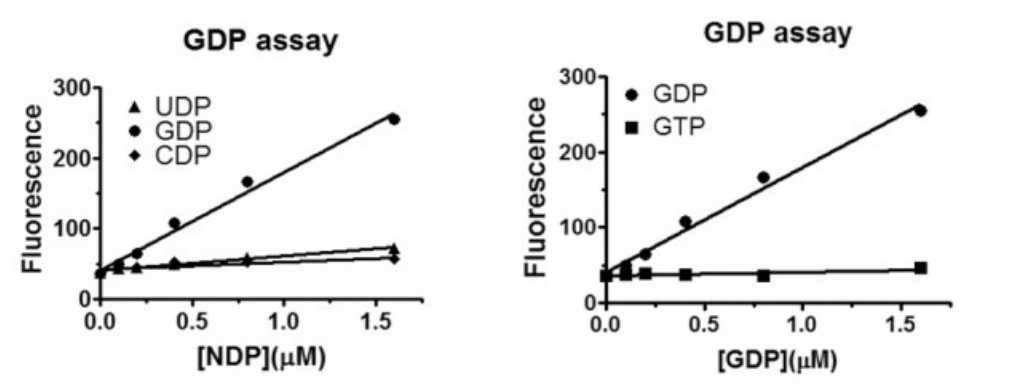
Kit components: 350 μl 10 x Buffer, 33 μl 100x MUD reagent 1, 33 μl 100x MUD reagent 2, 330 μl 10 x fluorescence dye, 30 μl 1 mM UDP.

MicroMolar UDP assay kit - 500 assays (Catalog No. MUD500K)

Kit components: 1800 μl 10 x Buffer, 165 μl 100x MUD reagent 1, 165 μl 100x MUD reagent 2, 1800 μl 10 x fluorescence dye, 100 μl 1 mM UDP.

GDP

The MicroMolar GDP Assay is for measurement of GDP concentrations in a sub-micromolar to low micromolar range. UDP and CDP showed very little background. ADP will also be detected. Nucleoside triphosphates (ATP, UTP, GTP and CTP) and monophosphates (AMP, UMP, GMP and CMP) are not detected. The GDP assay is based on fluorescence measurement with emission at 535 nm and excitation at 485 nm. It is a high throughput assay using regular black 96-well or 384-well plates. Micro-cuvettes may also be used for detection of the fluorescence signals. The assay can be used to measure contamination of GDP in GTP samples or monitor hydrolysis of GTP. It can also be used for measurement of enzyme activities that generate GDP molecules. The GDP assay is compatible with common reaction buffers with magnesium. It is not compatible with samples containing ADP or high concentrations of DNA or RNA.



MicroMolar GDP assay kit -100 assays (Catalog No. MGD100K)

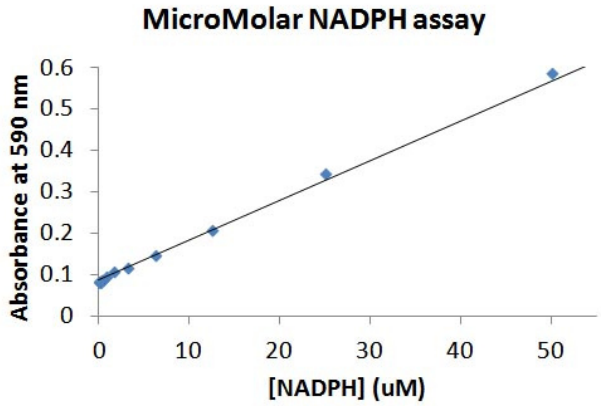
Kit components: 350 μl 10 x Buffer, 33 μl 100x MGD reagent 1, 33 μl 100x MGD reagent 2, 330 μl 10 x fluorescence dye, 30 μl 1 mM GDP.

MicroMolar GDP assay kit -500 assays (Catalog No. MGD500K)

Kit components: 1800 μl 10 x Buffer, 165 μl 100x MGD reagent 1, 165 μl 100x MGD reagent 2, 1800 μl 10 x fluorescence dye, 100 μl 1 mM GDP.

2.1.6 NADPH

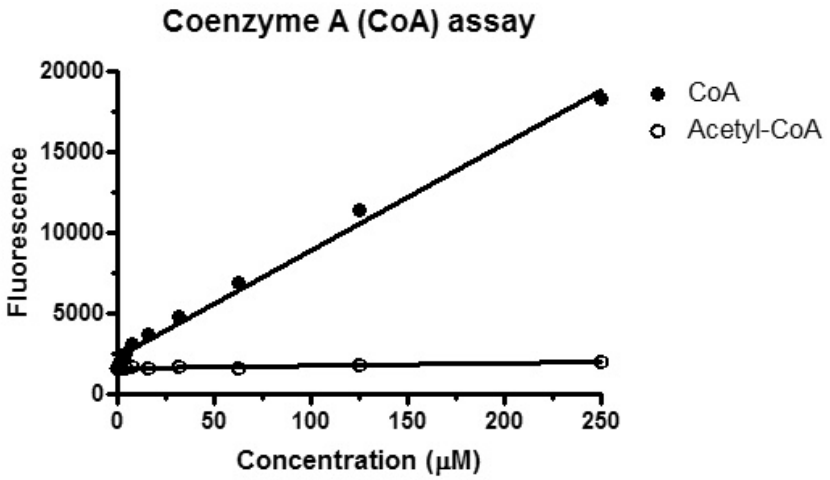
The assay measures micromolar concentrations of NADPH. NADPH is the reduced form of nicotinamide adenine dinucleotide phosphate (NADP). NADPH is an enzymatic cofactor for many reductases and acts as an electron donor. It is a product of many oxidases using NADP as an electron acceptor. Both NADPH and NADP are involved in many biosynthesis and degradation pathways. The MicroMolar NADPH Assay Kit provides a convenient tool for sensitive detection of NADPH. The assay is based on detection of light absorbance at 590 nm and the assay process is completed within 20 min. It is in a 96-well plate format and can be used for high throughput screening of enzymes involving NADPH. The assay is compatible with most common buffers in biochemistry labs. It is not compatible with NADH or other reducing reagents such DTT.



The **MicroMolar NADPH Assay Kit (Catalog No. NADPH100)** includes 1 ml of 10x Assay buffer, 0.1 ml of 20 x Reagent A, 0.1 ml of 20 x Reagent B, 1 ml of 10x Reagent C and 0.020 ml of 10 mM NADPH. It is for 100 assays in a 96-well plate format.

2.1.7 Coenzyme A (CoA)

The Coenzyme A Assay Kit is for measurement of micromolar concentrations of Coenzyme A (CoA). The assay is based on the principle that the thiol group of CoA interacts with the CAK reagent and enhances the fluorescence intensity at 535 nm (excitation at 485 nm). Acetyl-CoA does not interact with the CAK reagent.

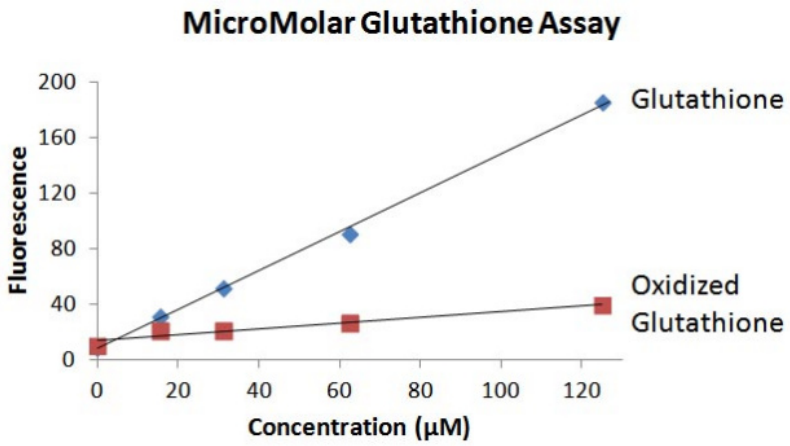


Coenzyme A Assay Kit (Catalog No CAK1000): The assay reagent is sufficient for measurement of 1000 samples using 384-well plates. The assay kit can be used for measurement of other thiols.

The assay is compatible with HEPES buffer, low concentrations of non-ionic detergent (<0.01%), sodium and potassium salt, $MgCl_2$ (< 5 mM), $CaCl_2$ (<5 mM) and phosphate (< 1 mM). The assay is not compatible with ammonium, Tris buffer or other primary amine buffers. It is not compatible with amino acids, DTT or EDTA.

2.1.8 Glutathione (GSH)

Glutathione is an essential antioxidant in the cells that prevents damage of biomolecules due to oxidation. Glutathione is also a popular ingredient in pharmaceutical products. The MicroMolar Glutathione Assay Kit is for measurement of micromolar concentrations of glutathione. The assay is based on increase of the fluorescence intensity (emission 535 nm, excitation 485 nm) of the kit fluorescence dye MAA upon binding to glutathione. The assay detects reduced glutathione (GSH) at a much higher sensitivity than the oxidized form (GSSG).

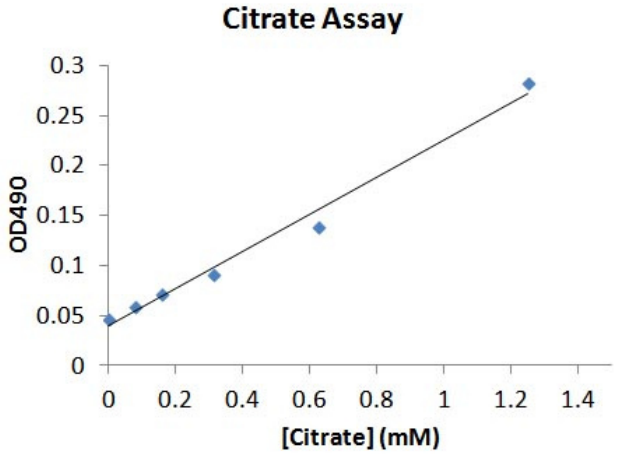


The assay kit can be used for measurements glutathione concentrations in pharmaceutical products, biochemical reactions or other samples. The assay is compatible with HEPES buffer, low concentrations of non-ionic detergent (<0.01%), $MgCl_2$ (< 5 mM), $CaCl_2$(<5 mM), EDTA (< 1 mM) and phosphate (< 1 mM). It is not compatible with thiol compounds such as DTT and cysteine. It is not compatible with samples with histidine.
The MicroMolar Glutathione Assay Kit (Catalog No MGA200) includes 500 µl of 10 x MAA dye. It is for 200 assays using 96-well plates.

2.1.9 Citrate

Citrate is an important biomolecule and popular additive in pharmaceutical products.
The Citrate Assay Kit is for measurement of sub-millimolar to low millimolar concentrations of

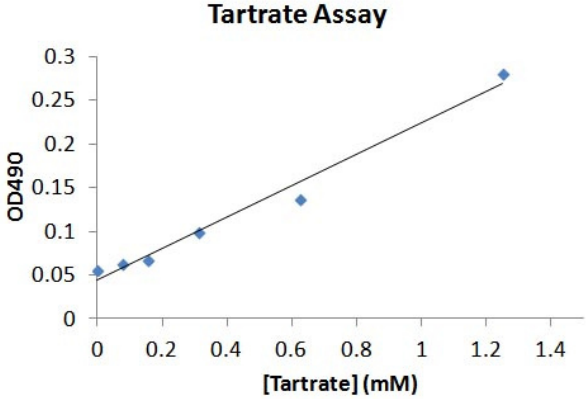
citrate. The assay is based on increase of the optical density at 490 nm (OD_{490}) of the kit reagent upon binding to citrate. The assay is compatible with Tris-HCl buffers. It is not compatible with phosphate buffers, polycarboxylic acids or other poly acids.



The Citrate Assay kit (Catalog No CIT100) includes 1.5 ml of Reagent A, 1.5 ml of 10 x Reagent B and 0.2 ml of 100 mM ammonium citrate. It is for 100 assays using 96-well plates. Cuvettes may also be used for measurements.

2.1.10 Tartrate

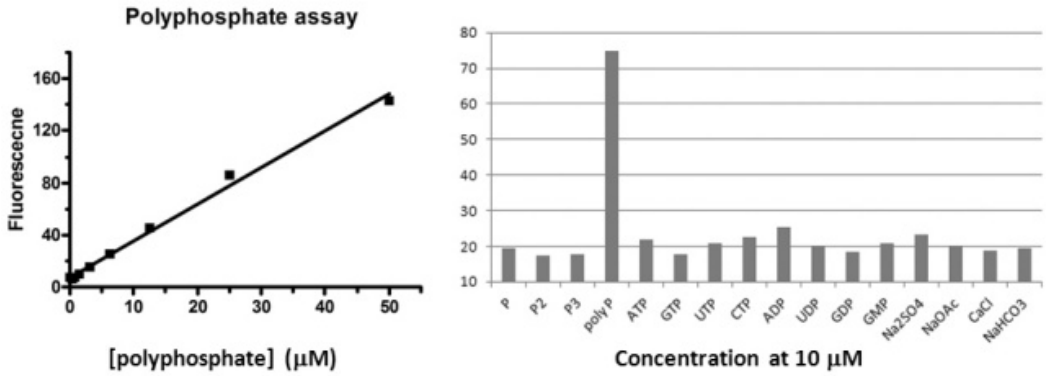
Tartrate is a common additive in pharmaceutical products and food products. The Tartrate Assay Kit is for measurement of sub-millimolar to low millimolar concentrations of tartrate. The assay is based on increase of the optical density at 490 nm (OD_{490}) of the kit reagent upon binding to tartrate. The assay is compatible with Tris-HCl buffers. It is not compatible with phosphate buffers, polycarboxylic acids or other poly acids.



The Tartrate Assay kit (Catalog No TAR100) includes 1.5 ml of Reagent A, 1.5 ml of 10 x Reagent B and 0.2 ml of 100 mM ammonium tartrate. It is for 100 assays using 96-well plates. Cuvettes may also be used for measurements.

2.1.11 Polyphosphate

Inorganic polyphosphate is a linear molecule composed of tens or hundreds of phosphate residues linked together. In bacteria, polyphosphate kinase (PPK) converts polyphosphate and ADP to ATP. The MicroMolar Polyphosphate Assay Kit is for measurement of micromolar concentrations of polyphosphate. The assay is based on increase of the fluorescence intensity (emission 550 nm, excitation 415 nm) of the kit fluorescence dye PPD upon binding to polyphosphate. The assay is compatible with regular buffers and various phosphate compounds including inorganic phosphate, pyrophosphate, ATP, ADP and AMP. The assay kit can be used for measurements of polyphosphate in biological samples or environmental water samples.

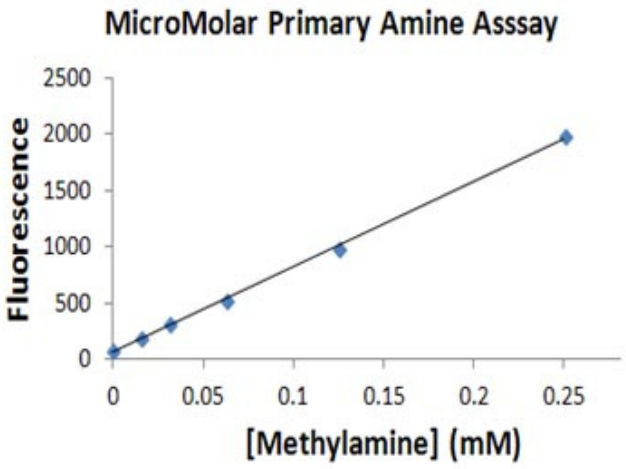


MicroMolar Polyphosphate Assay Kit (Catalog No: PPD1000)

The MicroMolar Polyphosphate Assay Kit is for measurement of micromolar concentrations of polyphosphate. The assay is based on increase of the fluorescence intensity (emission 550 nm, excitation 415 nm) of the kit fluorescence dye PPD upon binding to polyphosphate. The assay is compatible with regular buffers and various phosphate compounds including inorganic phosphate, pyrophosphate, ATP, ADP and AMP. The assay kit can be used for measurements of polyphosphate in biological samples or environmental water samples.

2.1.12 Primary amines

The MicroMolar Primary Amine Assay Kit is designed for measurement of primary amines or ammonia or their salts at micromolar concentrations. Amino acids, peptides and many drug molecules contain primary amine groups can be quantified using this kit. Protonated primary amines or ammonium salts release the free primary amine or ammonia in the kit assay buffer. Primary amines or ammonia interact with the assay reagent PAA dye to form fluorescent products with excitation at 390 nm and emission at 470 nm. The kit can be generally used for measurements of micromolar concentrations of primary amines or ammonia or their salts in biological samples, biochemical reactions, pharmaceutical products and environmental water samples. The assay is not compatible with amine-based buffers such as Tris buffer. It is compatible with thiol compounds such as DTT.



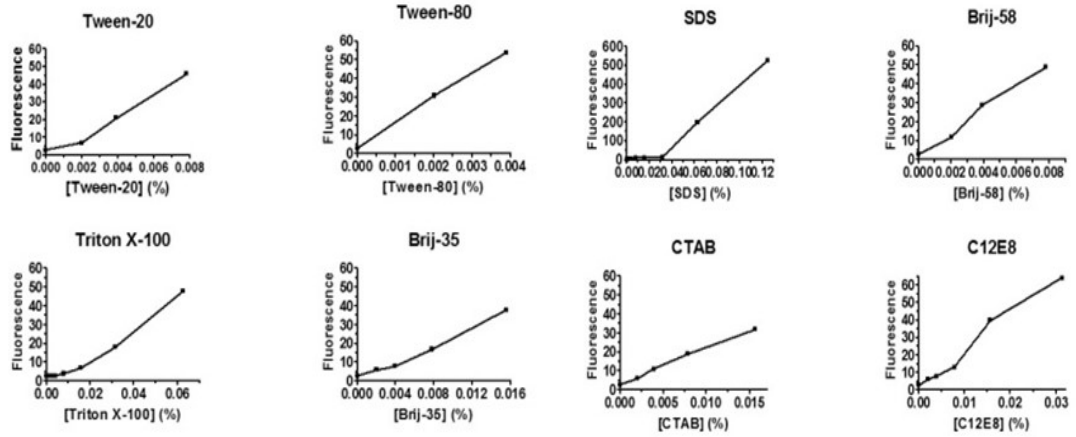
The **MicroMolar Primary Amine Assay Kit (Catalog No PAA100K)** includes 0.5 ml of 10 x PPA dye, 5.5 ml of Assay buffer, 5 ml of reagent A and 0.05 ml of 1 mM methylamine-HCl. It is for measurement of 100 samples using 96-well plates. Cuvettes may also be used for measurements.

2.2 Detergent and lipid assays

2.2.1 Detergent

The Detergent Assay Kit is for detection of various detergents at concentrations below their CMC values for most detergents. The concentration response is different from detergent to another. The assay is based on the principle that the detergent interacts with dye A43 and enhances the fluorescence intensity at 535 nm (excitation at 485 nm). The assay is compatible

with most buffers and salts. It is not compatible with phosphate. Molecules such as ATP or ADP that release phosphate may interfere with the assay.

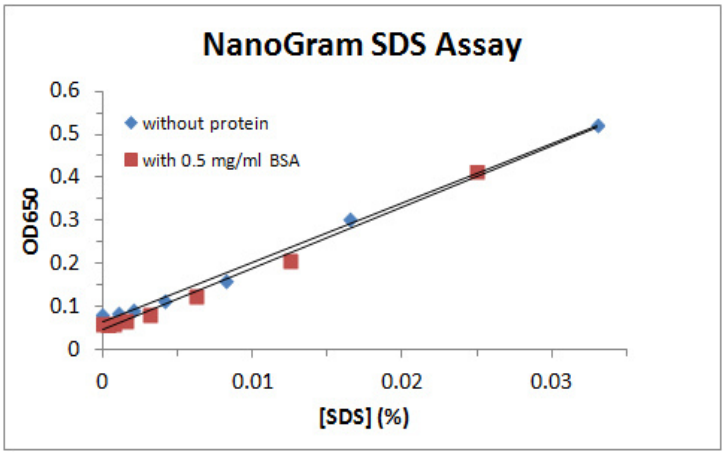


Detergent assay kit (Catalog No. DAK1000)

Kit components: 1 ml 100 x dye A43, 100 ml Reagent 1, 0.5 ml 100 x Reagent 2.

2.2.2 SDS

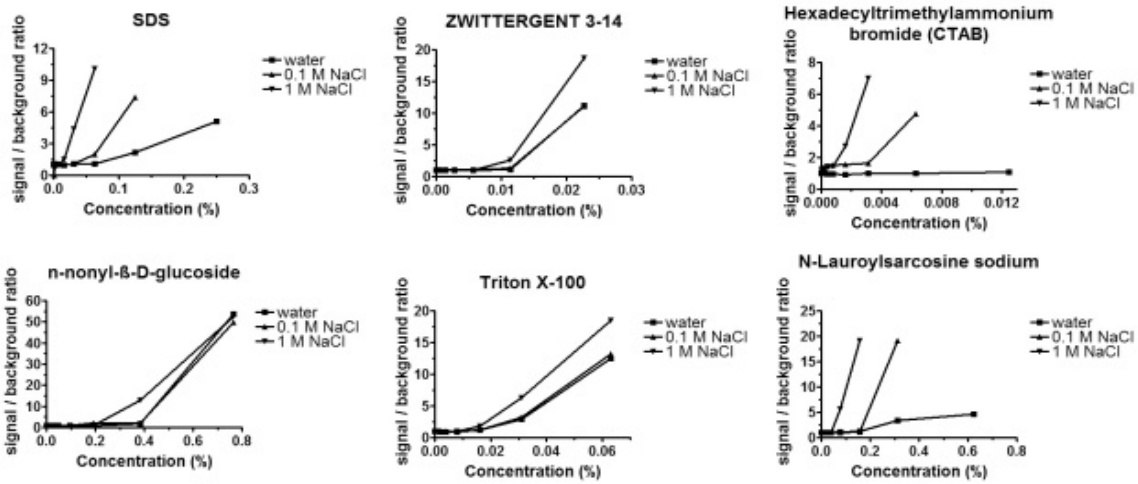
SDS (sodium dodecyl sulfate or sodium lauryl sulfate) is a common detergent and protein denaturant. In laboratories, SDS is broadly used in cell lysis, protein analysis (SDS-PAGE) and membrane protein folding studies. In industry, SDS is used as a highly effective surfactant and is a key component in many cleaning products. SDS strongly binds to proteins and changes the protein conformation that affects the protein function. Complete removal of SDS in a protein solution is essential to fully recover the protein function and stability. The NanoGram SDS Assay Kit (Catalog No SDS200) is designed for measurement of nanograms of SDS. The assay sensitivity is 0.002% SDS which is about 100 fold lower than its CMC value. The assay is based on increase of light absorbance at 650 nm of the dye MPS6 in the presence of SDS. The assay kit can be used for measurements SDS concentrations in samples with or without proteins. The assay is compatible with most biochemical buffers. It is not compatible with phosphate buffers. Inorganic phosphate interferes with the assay.



NanoGram SDS Assay Kit (Catalog No. SDS200)
Kit components: 20 ml MPS6 Dye.

2.2.3 Detergent CMC

The critical micelle concentration (CMC) value of a detergent depends on the solution compositions such as salt concentrations. Knowing the CMC value of the detergent in a particular buffer is essential in membrane protein extraction, purification and crystallization. Membrane proteins tend to aggregate when the detergent concentration is below the CMC. A too high detergent concentration may cause many problems including protein instability, difficulty in purification and crystallization. A low concentration of a detergent may be used for enzyme assays where formation of detergent micelles should be avoided in testing enzyme inhibitors. The CMC assay kit is based on the principle that the detergent interacts with the fluorescence dye and enhances the fluorescence intensity at 465 nm (excitation at 360 nm). The kit can be used for measurement of the CMC values of known detergents in solutions with different salt concentrations that affect the CMC values. It can also be used to measure the CMC values of new detergents or to test if a new molecule is a detergent that should have a CMC value.

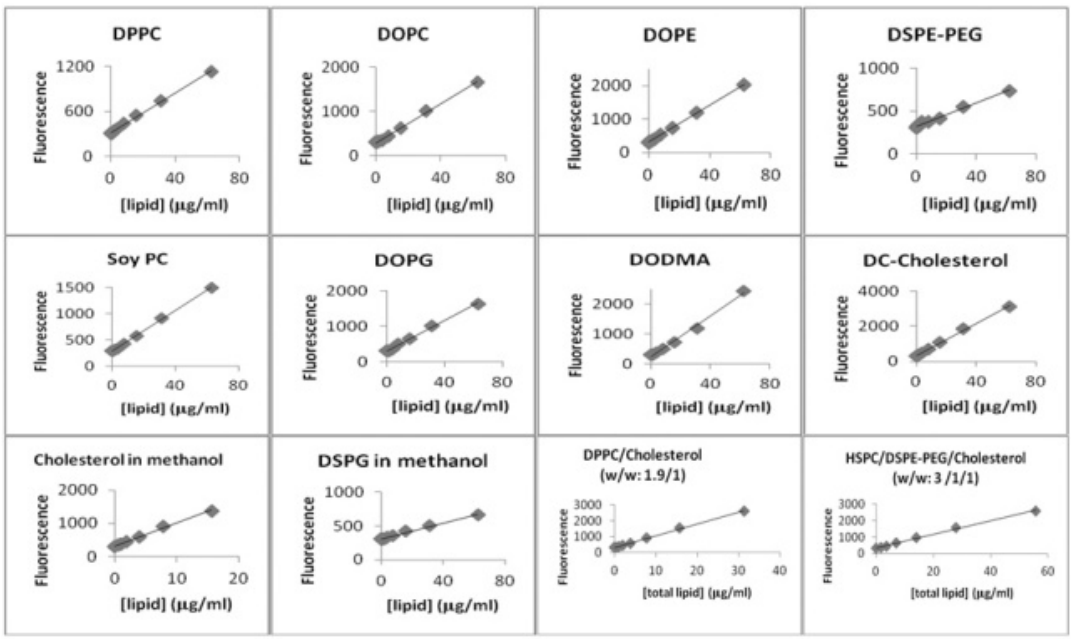


Detergent Critical Micelle Concentration (CMC) Assay Kit (Catalog No. CMC1000)

Kit components: 105 µl 1000 x CMC dye.

2.2.4 lipids

Lipids are essential components of cell membranes. Synthetic lipids or lipids isolated from the nature are used for constructions of bilayer membranes for various applications such as



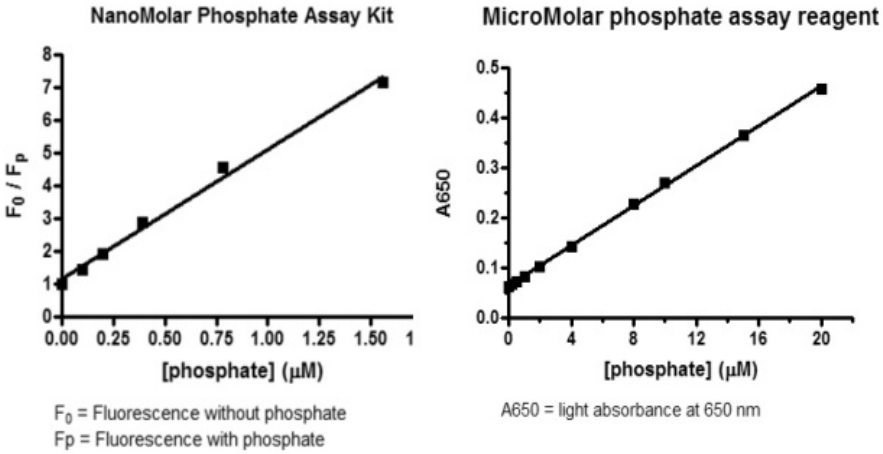
membrane protein reconstitution and liposomal drug formulations. The **MicroGram Lipid Assay Kit (Catalog number LIP1000)** is designed for measurement of various lipids at concentrations of micrograms per milliliter. The assay is based on measurement of fluorescence at 465 nm (excitation at 360 nm). It can be used to measure concentrations of various purified lipids or lipid mixtures such as cell membrane lipids or liposomes.

The **MicroGram Lipid Assay Kit (Catalog number LIP1000)** includes 0.1 ml of 1000 x LIP 10 Dye. It is for measurement of 1000 samples using 96-well plates. Cuvettes may also be used for measurements.

2.3 Buffer components

2.3.1 Phosphate

The **MicroMolar Phosphate Assay Reagent** is for measurement of 0.2 μM – 20 μM (micromolar) phosphate. The assay is based on the principle that phosphate interacts with molybdate and forms a blue complex with Malachite Green dye in an acidic solution. The phosphate amount is measured by detection of the light absorbance at 650 nm.



F_0 = Fluorescence without phosphate
Fp = Fluorescence with phosphate

A650 = light absorbance at 650 nm

MicroMolar Phosphate Assay Reagent (Catalog No. MPA3000)
Kit components: 450 ml Reagent.

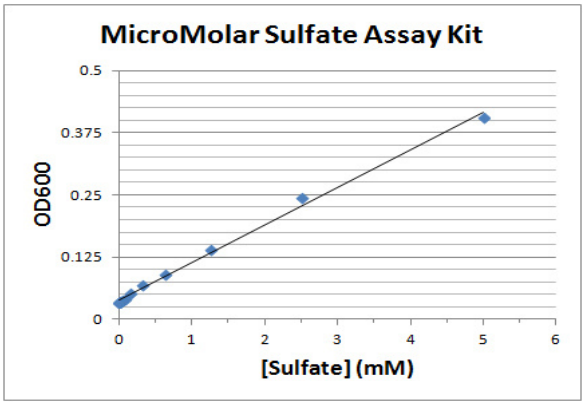
Measure low phosphate concentrations
The **NanoMolar Phosphate Assay Kit** is for measurement of sub-micromolar (< 1 μM) concentrations of phosphate. The assay is based on reduction of the fluorescence intensity of

the kit reagent by phosphate. It can be used for measurement of ATPase or GTPase activity of protein samples. It is suitable for high throughput screen (HTS) of drug targets with ATPase or GTPase activity. Since the assay is ultra-sensitive to phosphate, it is particularly useful to detect ATPase or GTPase activity where a limited level of phosphate is produced. The ATP or GTP concentration used for the assay should be as low as possible because ATP and GTP products contain some phosphate level. The ATP or GTP concentration should be below 50 μM. The assay is compatible with regular buffers with various concentrations of salts, glycerol (<5%), MgCl2, EDTA, Ethanol, DMSO and 0.005% CHAPS. Most detergents reduce the assay sensitivity. It is not compatible with DTT. TCEP-HCl (< 2 mM) instead of DTT may be used if a reducing reagent is necessary for an enzyme reaction (TCEP-HCl, Thermo Scientific, Catalog No 20490). The assay is compatible with 96-well plates (Costar 3915 and Greiner 655076), 384-well plates (Corning 3571, Matrix 4318) and low volume 384-well plates (Matrix 4363). It is not compatible with some plates such as Corning 3676 or Corning 3575.

NanoMolar Phosphate Assay Kit (Catalog No. NPA1000)
Kit components: 100 ml Reagent P1, 1 ml 100x Reagent P2, 0.1 ml 1 mM potassium phosphate.

2.3.2 Sulfate

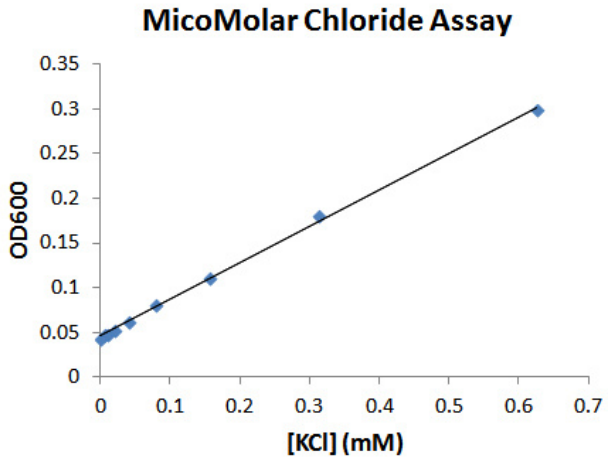
Sulfate plays key roles in metabolism of many important biomolecules including steroids, neurotransmitters, bile acids, glycosaminoglycans and cartilage proteoglycans. The MicroMolar Sulfate Assay Kit (Catalog No MSA200) is designed for measurement of sub-millimolar to millimolar concentrations of sulfate. The assay is based on increase of the light scattering at 600 nm of the sulfate precipitation with barium included in Reagent BP. The assay kit can be used for measurements of free sulfate concentrations in biological samples, biochemical reactions, pharmaceutical products and environmental water samples.



MicroMolar Sulfate Assay Kit (Catalog No. MSA200)
Kit components: 40 ml Reagent BP.

2.3.3 Chloride

Chloride plays a variety of important physiological roles in the cells. Chloride channels are responsible for cell membrane potential and regulating cell volume. The chloride level in neurons is related to the actions of neurotransmitters glycine and GABA . The chloride level in blood is directly related to the kidney functions. Chlorides are the most common reagents and buffers used in research and the most abundant anion in nature. The **MicroMolar Chloride Assay Kit (Catalog number CLA100)** provides a quick and simple method for quantification of chloride in a variety of samples. The kit does not contain toxic mercury component. It is based on measurement of optical density at 600 nm (OD_{600}) that correlates the chloride concentration in the presence of Reagent CLA. The assay linear range is 0.01 mM – 0.6 mM. Samples with higher chloride concentrations are diluted. The assay is compatible with a HEPES buffer. It is not compatible with EDAT or thiol compounds such as DTT, 2-mercaptoethanol or cysteine.

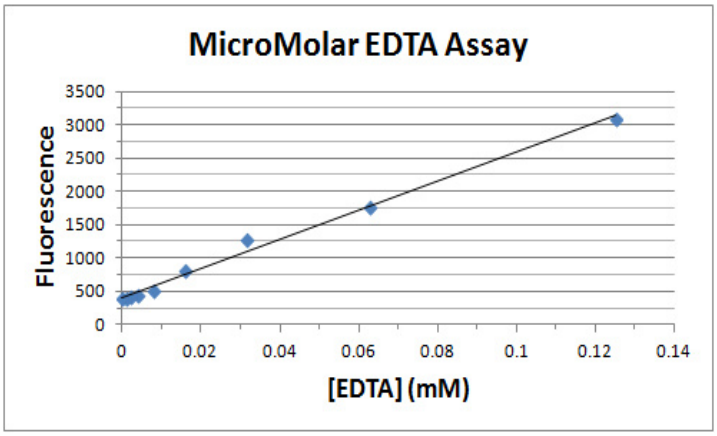


The **MicroMolar Chloride Assay Kit (catalog number CLA100)** includes 3 ml of Reagent CLA and 0.1 ml of 10 mM NaCl solution. It is for measurement of 100 samples using 96-well plates. Cuvettes may also be used for measurements.

2.3.4 EDTA

EDTA (Ethylenediaminetetraacetic acid) is a common chelating agent in biochemistry. EDTA should be avoided in protein purification with a Ni-column. Free EDTA should not be included

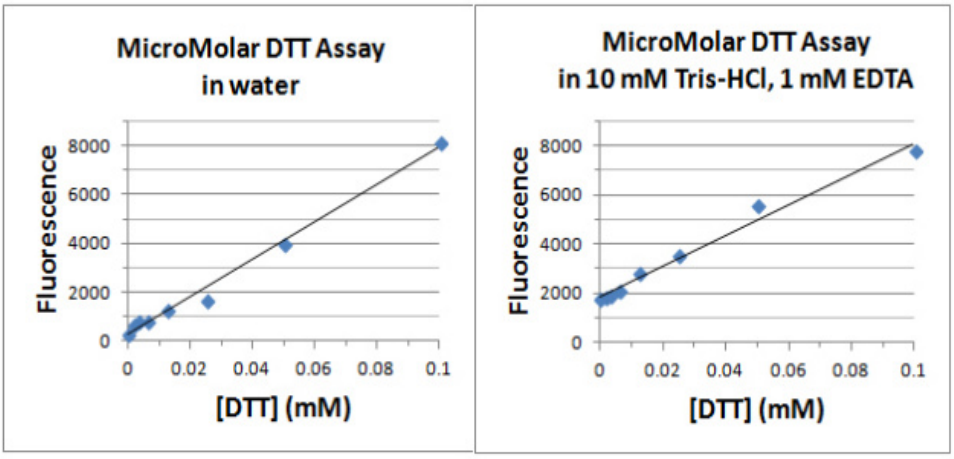
in many enzyme reactions where divalent cations such magnesium, calcium and zinc are required for the enzyme activity. The MicroMolar EDTA Assay Kit (Catalog No EDTA200) is designed for measurement of micromolar concentrations of EDTA. The assay is based on increase of fluorescence at 535 nm of the dye C56 in the presence of EDTA. The assay kit can be used for measurements EDTA concentrations in biological samples, biochemical reactions and environmental water samples. The assay is compatible with HEPES buffer, low concentrations of non-ionic detergent (<0.01%), Tris-HCl (<10 mM), and phosphate (< 1 mM). It is not compatible with thiol compounds such as DTT, 2-mercaptoethanol or cysteine.



MicroMolar EDTA Assay kit (Catalog No. EDTA200)
Kit components: 200 ul 100x C56 dye, 1 ml 1 mM EDTA.

2.3.5 DTT

DTT (Dithiothreitol) is a common reducing agent in biochemistry. Removal of DTT is required for Cys-based protein labeling and for disulfide bond formation in proteins. The MicroMolar DTT Assay Kit (Catalog No DTT200) is designed for measurement of micromolar concentrations of DTT. The assay is based on increase of fluorescence at 535 nm of the dye C55 in the presence of DTT. The assay kit can be used for measurements DTT concentrations in biological samples, biochemical reactions and environmental water samples. The assay is compatible with HEPES buffer, low concentrations of non-ionic detergent (<0.01%), $MgCl_2$ (< 5 mM), $CaCl_2$ (<5 mM), Tris-HCl (<10 mM), EDTA (< 1mM) and phosphate (< 1 mM). It is not compatible with thiol compounds such as 2-mercaptoethanol or cysteine.

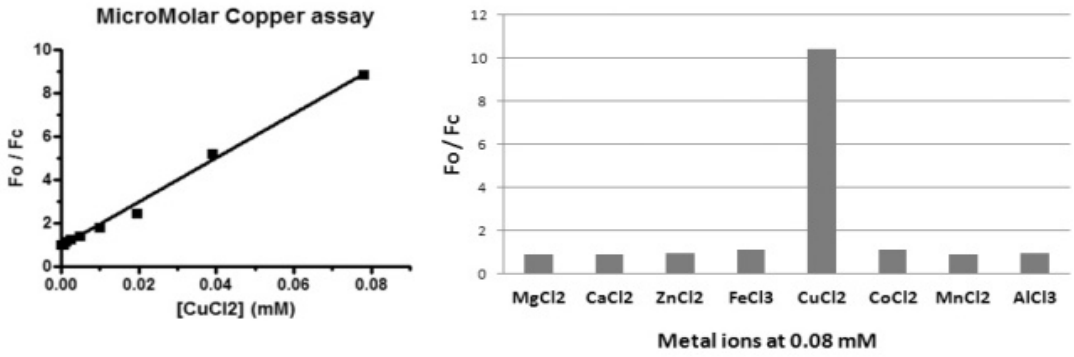


MicroMolar DTT Assay kit (Catalog No. DTT200)
Kit components: 5 ml Dye C55.

2.4 Metal ions

2.4.1 Copper

Copper (Cu^{2+}) is an essential metal ion in biological systems. Many enzymes such as amine oxidase and galactose oxidase require copper for their biological functions. Low levels of copper are associated with disorders including mental retardation, depigmentation, anaemia, hypotonia and scorbutic changes in bone. Levels of copper are key diagnostic indicator of diseases such as Wilson's disease, microcytic hypochromic anaemia and bone disease. The MicroMolar Copper Assay Kit is for measurement of micromolar concentrations of free copper



ion Cu^{2+} (0.001 mM – 0.050 mM). The assay is based on the principle that binding the fluorescence dye MCA selectively with copper results in decrease of the fluorescence intensity

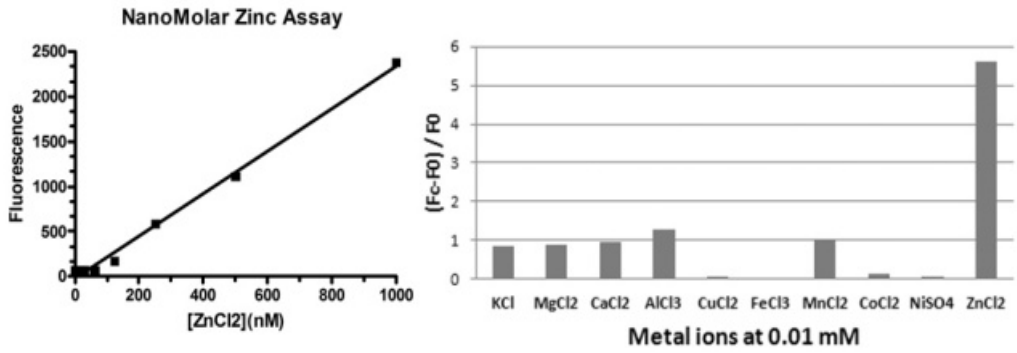
(emission 535 nm, excitation 485 nm). The assay is compatible with 1 mM Mg^{2+}, Ca^{2+}, Zn^{2+}, Mn^{2+} and Al^{3+}, 0.1 mM Co^{2+} and Ni^{2+}. The Cu^{2+} sample should be diluted in water or 10 mM HEPES buffer, pH 7.4. It is not compatible with Tris buffer. EDTA, thiols, ammonia and amines strongly binds Cu^{2+} and should be avoided in the assay.

MicroMolar Copper Assay Kit (Catalog No. MCA1000)

Kit components: 105 µl 1000 x MCA dye, 30 µl 1 mM $CuCl_2$.

2.4.2 Zinc

Zinc (Zn^{++}) is an essential metal ion in biological systems. Zinc is a cofactor of hundreds of enzymes and plays important roles in signal transduction, gene expression, regulation of apoptosis, synaptic plasticity and prostate gland function. Zinc deficiency is associated with malabsorption syndrome, chronic GI, liver disease, diabetes, renal disease, sickle cell disease, anorexia nervosa, and HIV infection. The NanoMolar Zinc Assay Kit is for measurement of submicromolar concentrations of zinc (0.1 µM – 2 µM). The assay is based on the principle that binding the fluorescence dye NZA selectively with Zinc results in increase of the fluorescence intensity (emission 535 nm, excitation 485 nm). The assay is compatible with regular buffers with different metal ions including 10 µM Mg^{2+}, Ca^{2+}, Cu^{2+}, Mn^{2+}, Al^{3+}, Fe^{3+}, Ag^{+}, Co^{2+} and Ni^{2+}. Chelators such as EDTA and thiol compounds bind zinc and should be avoided in the assay. The assay kit can be used for high-throughput measurements of zinc concentrations in biochemical assay reactions associated with zinc metabolism or environmental water samples.

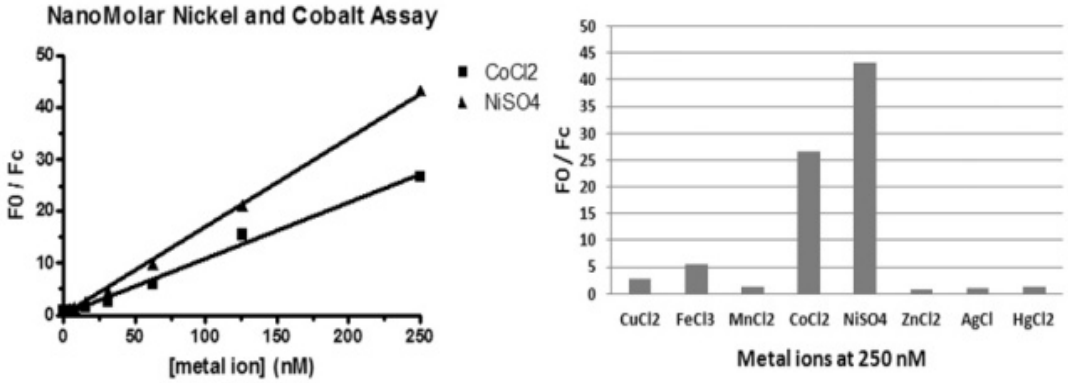


NanoMolar Zinc Assay Kit (Catalog No. NZA1000)

Kit components: 250 µl 100 x NZA dye.

2.4.3 Nickel and Cobalt

Nickel (Ni^{++}) and cobalt (Co^{++}) are essential metal ions in biological systems. Many enzymes such as methionine aminopeptidase and glucose isomerase contain cobalt. Some other enzymes such as ureases from bacteria and plants use nickel as a cofactor. Synthesis of Ni / Co enzymes and coenzyme B12 requires high-affinity uptake of the metal ions from natural environments. In bacteria, Ni and Co uptake is mediated by secondary transporters and ATP-binding cassette systems. Understanding the differences between cobalt and nickel transporters might lead to drug development for gastritis and peptic ulceration. The NanoMolar Nickel / Cobalt Assay Kit is for measurement of nanomolar concentrations of nickel or cobalt (1 nM – 200 nM). The assay is based on the principle that binding the fluorescence dye NMA with nickel or cobalt ions results in decrease of the fluorescence intensity (emission 535 nm, excitation 485 nm). Other metal ions including Mg^{2+}, Ca^{2+}, Zn^{2+}, Mn^{2+}, Al^{3+}, Cu^{2+} and Ag^{+} give much lower assay sensitivity. Chelators such EDTA and thiol compounds bind strongly some of the metal ions and should be avoided in the assay.



NanoMolar Nickel / Cobalt Assay Kit (Catalog No. NMA1000)

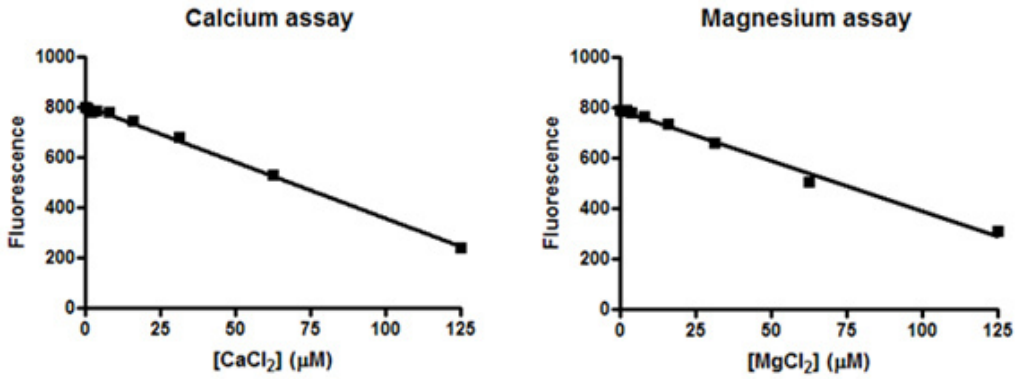
Kit components: 330 µl 100 x NMA dye.

2.4.4 Calcium and magnesium

Calcium (Ca^{2+}) or Magnesium (Mg^{2+}) is an essential metal ion in biological systems. These divalent ions are also common components in biochemical buffers and pharmaceutical products. The MicroMolar Calcium / Magnesium Assay Kit is for measurement of micromolar concentrations of calcium, and magnesium (10 µM – 120 µM). The assay may also be used to

detect other divalent metal ions. The assay is based on the complex formation of the divalent metal ion with the assay reagent that results in reduction of the fluorescence intensity (emission 535 nm, excitation 485 nm). Chelators such EDTA and thiol compounds bind divalent ions and should be avoided in the assay. The assay is compatible with HEPES buffer, low concentrations of non-ionic detergent (<0.01%), Tris-HCl (<10 mM), and phosphate (< 1 mM). It is not compatible with thiol compounds such as DTT, 2-mercaptoethanol or cysteine. It is interfered by divalent metal ions such as Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, $Hg^{2+}$and Zn^{2+}. For specific assays of transitional metal ions such as $Cu^{2+}$and Zn^{2+}, please visit http://www.profoldin.com/concentration.html.

The assay kit can be used for measurements calcium or magnesium concentrations in buffer samples or pharmaceutical products.

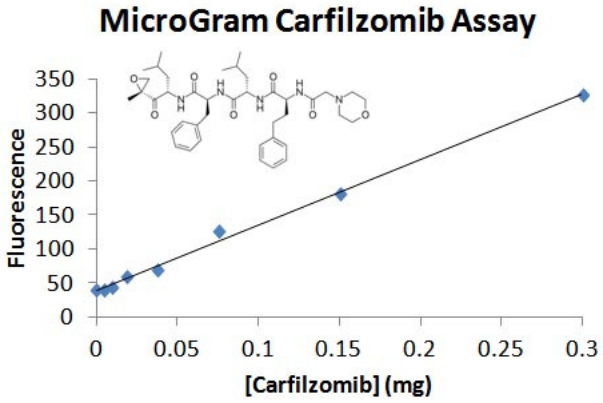


The **MicroMolar Calcium / Magnesium Assay Kit (Catalog No DMA200)** includes 200 µl of 100 x C56 dye and 1000 µl of 10 x Reagent E and 50 µl of $CaCl_2$. It is for measurement of 200 samples using 96-well plates. Cuvettes may also be used for measurements.

2.5 Drug molecules

2.5.1 Carfilzomib

Carfilzomib is an anti-cancer drug. It is a peptide derivative that selectively inhibits the activity of the 20S proteasome. The MicroGram Carfilzomib Assay Kit is designed for measurement of micrograms of carfilzomib. The assay is based on increase of fluorescence at 535 nm of the assay reagent C33 in the presence of carfilzomib. The assay kit can be used for measurement of carfilzomib concentrations in drug discovery and development.

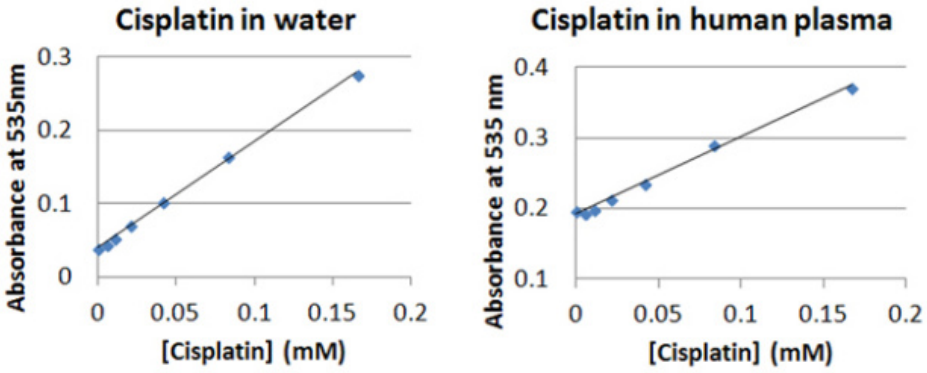


Due to the insolubility of carfilzomib in water, measurement of carfilzomib concentration is performed in ethanol. The assay is not compatible with thiol compounds or metal chelators such EDTA.

The **MicroGram Carfilzomib Assay Kit (Catalog No CFZ200)** includes 1 ml of 10 x C33 dye for 200 assays using 96-well plates. Cuvettes may also be used for the assay.

2.5.2 Cisplatin

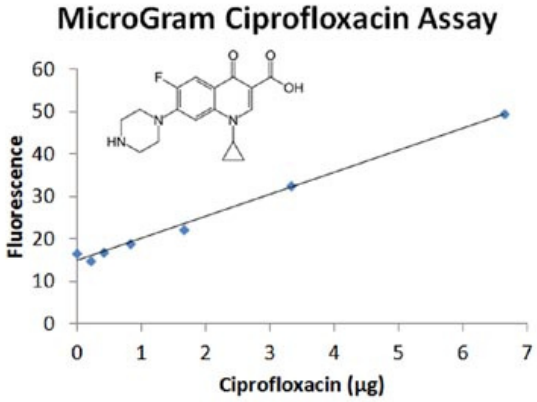
Cisplatin [or cis-diamminedichloroplatinum, $Pt(NH_3)_2Cl_2$] is the first member of platinum-containing anti-cancer drugs. It binds DNA and causes DNA crosslinking which ultimately triggers apoptosis (programmed cell death). The MicroMolar Cisplatin Assay Kit (Catalog No CPT200) is designed for throughput measurement of micromolar concentrations of Cisplatin. The assay is based on the light absorbance at 535 nm. The assay kit can be used for assays of cisplatin in drug discovery, drug development, pharmaceutical samples and biological samples.



The MicroMolar Cisplatin Assay Kit (Catalog No CPT200) includes 4 ml of Reagent A, 0.080 ml of 50 x Reagent B, and 2 ml of 10 x Reagent C. It is for 200 assays using 96-well plates. Cuvettes may also be used for measurements.

2.5.3 Ciprofloxacin

Ciprofloxacin is an antibiotic that binds DNA topoisomerases and blocks the DNA replication process in bacteria. The MicroGram Ciprofloxacin Assay Kit is designed for measurement of low micrograms of ciprofloxacin. The assay is based on increase of fluorescence at 470 nm of the assay reagent in the presence of ciprofloxacin. The assay kit can be used for measurement of ciprofloxacin concentrations in drug discovery, drug development and pharmaceutical samples. The assay is not compatible with biological samples containing amino acids or other molecules or buffers with amines.

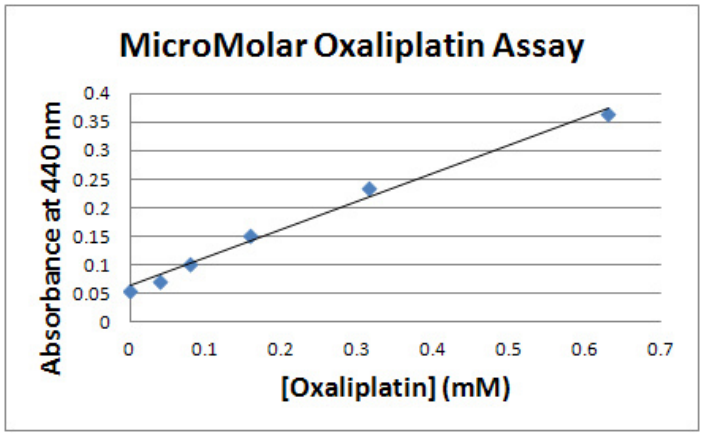


The **MicroGram Ciprofloxacin Assay Kit (Catalog No CIP100)** includes 1 ml of 10 x CIP dye, 10 ml of Assay buffer, 10 ml of reagent A. It is for measurement of 100 samples using 96-well plates. Cuvettes may also be used for measurements.

2.5.4 Oxaliplatin

Oxaliplatin assay

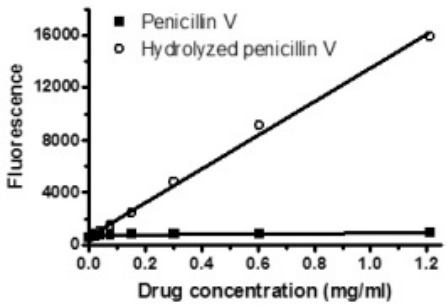
Oxlaiplatin is one of the platinum-based anti cancer drugs. It is used for treatment of colorectal cancer through its cytotoxic effects by inhibition of DNA synthesis in cells. The MicroMolar Oxaliplatin Assay Kit (Catalog No OPT200) is designed for high throughput measurement of micromolar concentrations of Oxaliplatin. The assay is based on the light absorbance at 440 nm. The assay kit can be used for assays of oxaliplatin in drug discovery, drug development, pharmaceutical samples and biological samples.

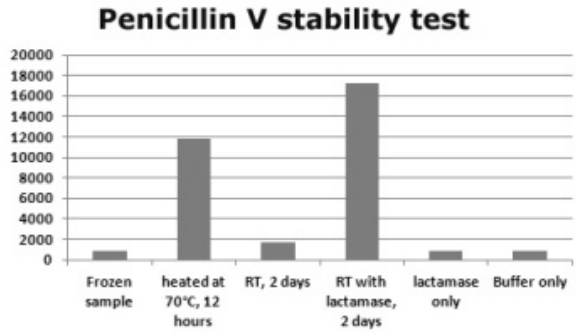


The **MicroMolar Oxaliplatin Assay Kit (Catalog No OPT200)** includes 4 ml of Reagent A, 0.080 ml of 50 x Reagent B, and 2 ml of 10 x Reagent C. It is for 200 assays using 96-well plates. Cuvettes may also be used for measurements.

2.5.5 Penicillin

Penicillin drugs such as Penicillin G and Penicillin V can be hydrolyzed to form penicilloic acid during storage or transportation due to various reasons including the following: (1) the temperature is too high; (2) the solution pH is not optimal for stabilization; or (3) the sample contains lactamase. The hydrolyzed penicillin drug molecule is thermodynamically more stable than the non-hydrolyzed one but completely inactive.





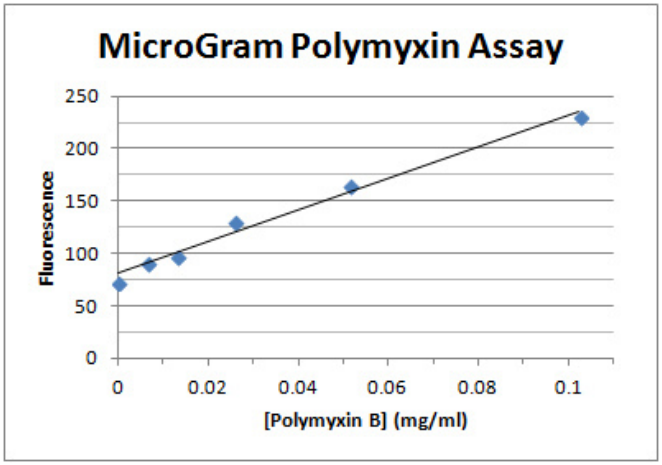
Penicillin Drug Stability Test Kit (Catalog No PST100)

The Penicillin Drug Stability Test Kit (Catalog No PST100) provides a reagent that quickly detects the hydrolyzed form of penicillin drugs including the commonly used Penicillin G and Penicillin V. The reagent, Dye PST, interacts with alpha-amino acids which are the hydrolysis products of penicillin drugs and generates fluorescence at 535 nm with excitation wavelength at 485

nm. The background signal with the non-hydrolyzed penicillin drugs is very low. The kit can be used to detect hydrolysis of penicillin drugs. It can also be used to detect the lactamase activity using penicillin as a substrate.

2.5.6 Polymyxin

Polymyxins are polypeptide antibiotics that bind lipopolysaccharide (LPS) and disrupt bacterial membranes. They are used for the treatment of Gram-negative bacterial infections. Since polymyxins increase permeability of the bacterial membrane system, they are also used for enhanced release of secreted toxins from bacteria. The MicroGram Polymyxin Assay Kit (Catalog No MPX200) is designed for measurement of microgram/ ml concentrations of polymyxins. The assay is based on increase of fluorescence at 535 nm of the assay reagent in the presence of polymyxins. The assay kit can be used for measurement of polymyxin concentrations in drug discovery, drug development and pharmaceutical samples.

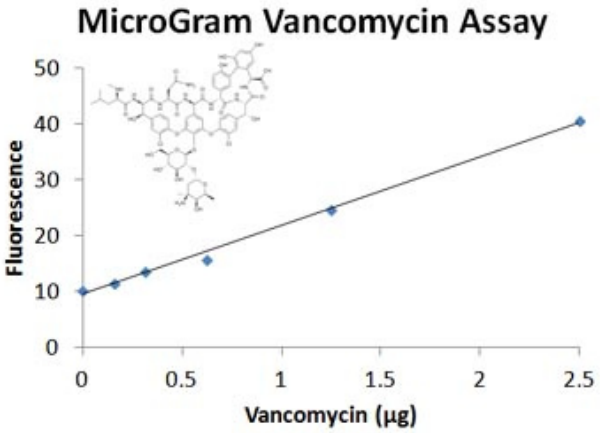


The **MicroMolar Polymyxin Assay Kit (Catalog No MPX200)** provides the reagent for measurement of 200 samples using 96-well plates. Cuvettes may also be used for measurements.

2.5.7 Vancomycin

Vancomycin is a glycopeptide antibiotic that blocks the cell wall synthesis of bacteria.
The MicroGram Vancomycin Assay Kit is designed for measurement of low micrograms of vancomycin. The assay is based on increase of fluorescence at 470 nm of the assay reagent in the presence of vancomycin. The assay kit can be used for measurement of vancomycin concentrations in drug discovery, drug development and pharmaceutical samples. The assay is

not compatible with biological samples containing amino acids or other molecules or buffers with amines.



The **MicroGram Vancomycin Assay Kit (Catalog No VAN100)** includes 0.5 ml of 10 x VAN dye, 5.5 ml of Assay buffer, 5 ml of reagent A. It is for measurement of 100 samples using 96-well plates. Cuvettes may also be used for measurements.

Chapter 3

Liposome and nanodisc products

Using ProFoldin's nano technology, various liposomes and nanodiscs can be produced in scales suitable for all research projects and industrial production. ProFoldin offers the following liposome products:

- Various tools for liposome preparation, purification and analysis
- Empty liposomes ready for loading various drugs using different loading mechanisms.
- Liposomes encapsulated with selected drugs for research.
- Liposomes encapsulated fluorescence dyes or biomarkers for tracking
- Liposomes for membrane protein reconstitution

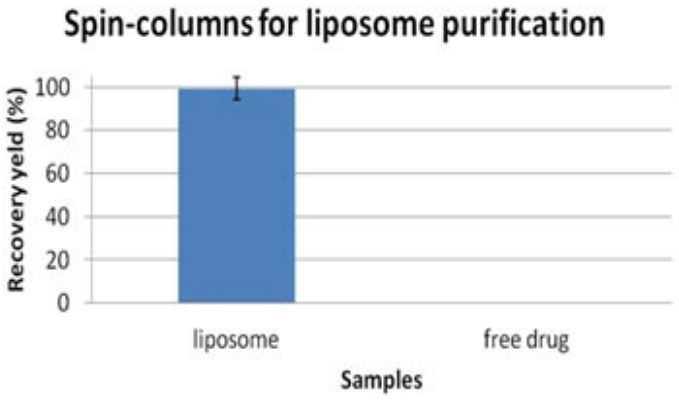
ProFoldin's SMA copolymers are specifically for nanodisc formation and have the following features:

- Have the styrene: maleic acid ratios 2:1 or 3:1 that are the best for membrane proteins.
- Have the molecular weight 8KDa and 10kDa that is proven good for nanodisc formation.
- Are pre-hydrolyzed and ready to be dissolved in common buffers.
- Form a solution optimal for nanodisc formation without the need of pH adjustment.

3.1 Liposome purification and analytic tools

3.1.1 Liposome purification kit

The **Spin-columns for Liposome Purification (Catalog No SLP20)** is designed for quick removal of free or non-encapsulated drugs. It removes > 99 % of the non-encapsulated drugs in a few minutes. Removal of the non-encapsulated drugs is based on a combination effect of



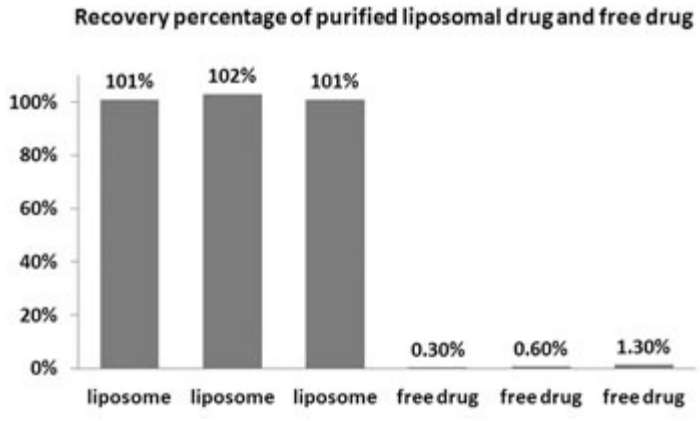
absorbance and size exclusion. When a purified 2 mg/ml liposomal doxorubicin is loaded, 99.3% liposomal doxorubicin is recovered. When a 2 mg/ml free doxorubicin is loaded, 0.2 % free doxorubicin is recovered.

The **Spin-columns for Liposome Purification** (Catalog No SLP20) includes 20 pre-packed spin columns. The columns are pre-packed in water and specifically designed for removal of free drugs in a liposome sample. Removal of the free drug is nearly complete. A minimal amount of liposomes may also bind to the column. For liposomal drug encapsulation and liposomal drug dissolution tests, please use assay kits specifically designed for drug encapsulation assays (Catalog No LDE10) or drug dissolution assays (Catalog No LDD10).

3.1.2 Drug encapsulation percentage assay kit

Liposome-formulated drug samples may contain free (non-encapsulated) drug molecules that are not encapsulated within the liposomes. Encapsulated drugs may leak out of liposomes during storage or due to exposure of the liposomes to organic solvent, ultrasound vibration or freezing or elevated temperatures. During production of liposomal drugs, drug loading may not be complete leaving certain percentage of the drug non-encapsulated. The **Liposome Drug Encapsulation Assay kit (Catalog No LDE10)** is designed to analyze the percentage of drug encapsulation in liposomes.

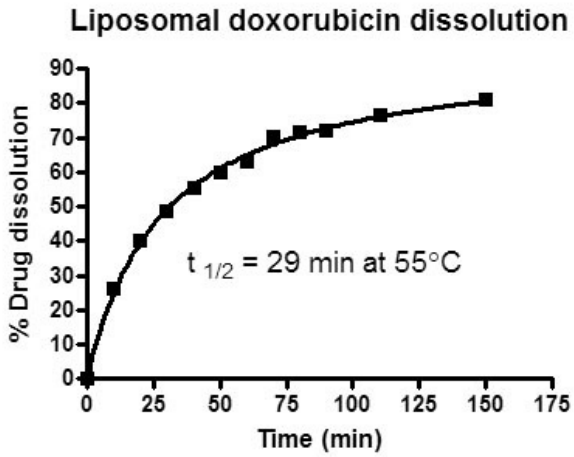
The kit is based on spin-column separation of the liposomes from the non-encapsulated drug molecules. It recovers 100 % ±2 % liposomes and removes > 98% non-encapsulated drugs. The complete separation between the liposomes and the non-encapsulated drug is based on a combination effect of absorbance and size between liposomes and non-encapsulated drug molecules.



The **Liposome Drug Encapsulation Assay kit (Catalog No LDE10)** includes 30 pre-packed spin-columns and 10 ml of elution buffer. It is for measurement of drug encapsulation of 10 liposome samples.

3.1.3 Liposomal drug dissolution assay kit

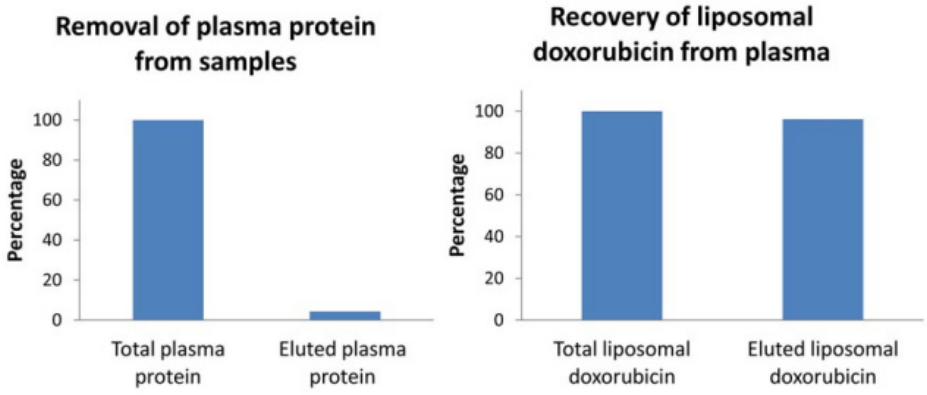
Liposome drug dissolution is release of the encapsulated drug into the medium of the liposome. The in vitro liposomal drug dissolution test is performed under certain chemical and physical pressure to simulate the release of the encapsulated drug. For liposomes with encapsulated drugs by pH gradient remote loading, ammonium salt is added in the medium to accelerate the drug release. The **Liposome Drug Dissolution Assay kit(Catalog No LDD05)** is designed to analyze liposome drug dissolution under optimized conditions. The released drug is removed by spin-column and the intact liposome is quantified.



The **Liposome Drug Dissolution Assay kit(Catalog No LDD05)** includes 40 pre-packed spin columns, 1.5 ml of 10 x dissolution buffer and 10 ml of elution buffer. It is for 5 drug dissolution assay tests of liposomal drug samples at the selected temperature.

3.1.4 Liposome plasma stability test kit

The **Liposome Plasma Stability Test Kit (Catalog No SPS20)** is designed for study stability of liposomal drugs in human or animal plasma or serum. Ready-to-use spin columns are employed for separation of liposomal drugs from non-encapsulated drugs and drugs that binds plasma proteins. After a quick spin-column process, more than 95 % of plasma proteins together with the or free drugs stay on the column. The intact liposomal drugs are in the elute. For example, the recovery yield of the intact liposomal doxorubicin was 96 % after incubation of the liposomal drug with human plasma at 37°C for 2 hours.



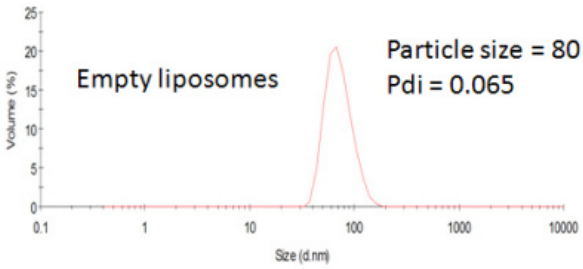
The Liposome Plasma Stability Test Kit (Catalog No SPS20) includes 20 pre-packed spin columns for analysis of 20 samples.

3.2 Ready-to-load liposomes for drug delivery

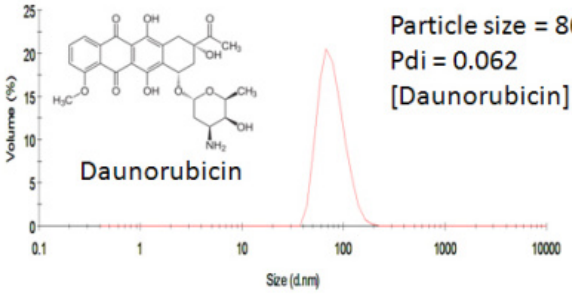
3.2.1 PEGylated liposomes with ammonium sulfate

Liposomal drug formulations provide great opportunities of improving the drug efficacy and toxicity profiles. Drug molecules with amine groups such as doxorubicin, daunorubicin,

Ready-to load PEGylated hydrogenated soy PC liposomes



Daunorubicin encapsulated in PEGylated hydrogenated soy PC liposomes



topotecan and irinotecan etc. can be loaded by ammonium-based pH gradient. The **Ready-to-load PEGylated HSPC Liposomes with Ammonium Sulfate (Catalog No PHPC200AS)** are high quality PEGylated liposomes that are ready to load drug molecules containing amine groups. The drug loading process is completed in about 3 hours after mixing the drug with the liposomes.

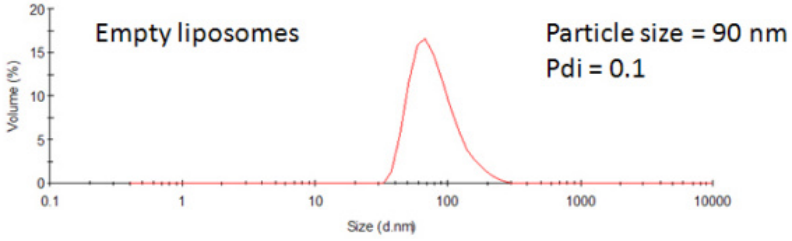
The **Ready-to-load PEGylated HSPC Liposomes with Ammonium Sulfate (Catalog No PHPC200AS)** includes 10 ml liposomes with 20 mg/ml lipid concentration. The mass ratio of lipids is HSPC: cholesterol: DSPE-PEG2000 = 3:1:1. The concentration of ammonium sulfate encapsulated within the liposomes for drug loading is 200 mM. The buffer is 10 mM histidine, pH 6.5, 9.2 % sucrose. Liposomes are stored in a 2°C to 8°C refrigerator. DO NOT freeze liposomes.

3.2.2 DPPC liposomes with ammonium sulfate

Liposomal drug formulations provide great opportunities of improving the drug efficacy and toxicity profiles. Drug molecules with amine groups such as doxorubicin, daunorubicin, topotecan and irinotecan etc. can be loaded by ammonium-based pH gradient. The **Ready-to-load DPPC Liposomes with Ammonium Sulfate (Catalog No DPC100AS)** are high quality non-

PEGylated liposomes that are ready to load drug molecules containing amine groups. The drug loading process is completed in about 3 hours after mixing the drug with the liposomes. The composition of the liposomes is DPPC and cholesterol in the weight ratio of 1.9:1. The total lipid concentration is 10 mg/ml. The liposomes are encapsulated with ammonium sulfate for drug remote loading. The average size of liposomes is about 90 nm with poly dispersity index (dpi) of 0.1.

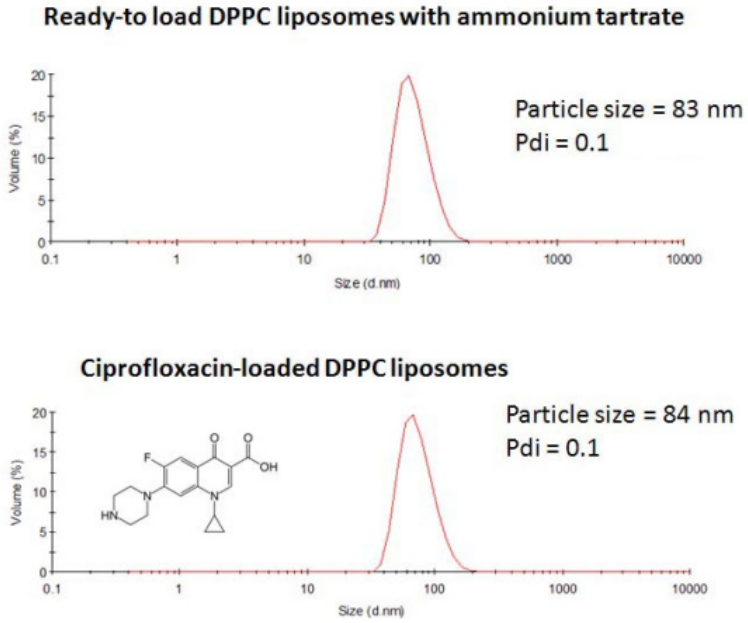
Ready-to load DPPC liposomes with ammonium sulfate



The **Ready-to-load DPPC Liposomes with Ammonium Sulfate (Catalog No DPC100AS)** includes 10 ml liposomes with 10 mg/ml lipid concentration. The mass ratio of lipids is DPPC: cholesterol = 1.9:1. The concentration of ammonium sulfate encapsulated within the liposomes for drug loading is 200 mM. The buffer is 10 mM histidine, pH 6.5, 9.2 % sucrose. Liposomes are stored in a 2°C to 8°C refrigerator. DO NOT freeze liposomes.

3.2.3 DPPC liposomes with ammonium tartrate

Liposomal drug formulations provide great opportunities of improving the drug efficacy and toxicity profiles. Drug molecules with amine groups such as doxorubicin and ciprofloxacin, etc. can be loaded by ammonium-based pH gradient. The **Ready-to-load DPPC Liposomes with Ammonium Tartrate (Catalog No DPC100AT)** are high quality non-PEGylated liposomes that are ready to load drug molecules containing amine groups. The drug loading process is completed in about 3 hours after mixing the drug with the liposomes. The composition of the liposomes is DPPC and cholesterol in the weight ratio of 1.9:1. The total lipid concentration is 20 mg/ml. The liposomes are encapsulated with ammonium tartrate for drug remote loading. The average size of liposomes is about 80 nm with poly dispersity index (dpi) of 0.1.

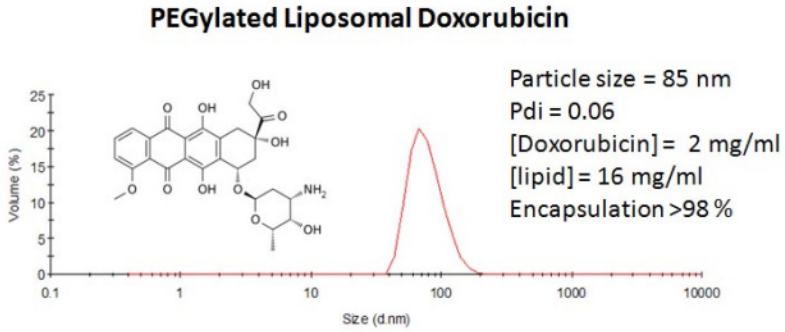


The **Ready-to-load DPPC Liposomes with Ammonium Tartrate (Catalog No: DPC100AT)** includes 10 ml liposomes with 10 mg/ml lipid concentration. The mass ratio of lipids is DPPC: cholesterol = 1.9:1. The concentration of ammonium tartrate encapsulated within the liposomes for drug loading is 300 mM. The buffer is 10 mM histidine, pH 6.5, 9.2 % sucrose. Liposomes are stored in a 2°C to 8°C refrigerator. DO NOT freeze liposomes.

3.3 Drug-loaded liposomes

3.3.1 Liposomal doxorubicin

The PEGylated liposomal doxorubicin includes the same concentrations of doxorubicin and lipids as that of clinical drugs Doxil or Lipodox but for laboratory research use only. The liposomal doxorubicin contains 2 mg/ml doxorubicin and 16 mg/ml lipids composed of 9.6 mg/ml hydrogenated soy phosphocholine, 3.2 mg/ml 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000]and 3.2 mg/ml cholesterol. The average size of liposomes is about 85 nm with poly dispersity index (dpi) of below 0.1.

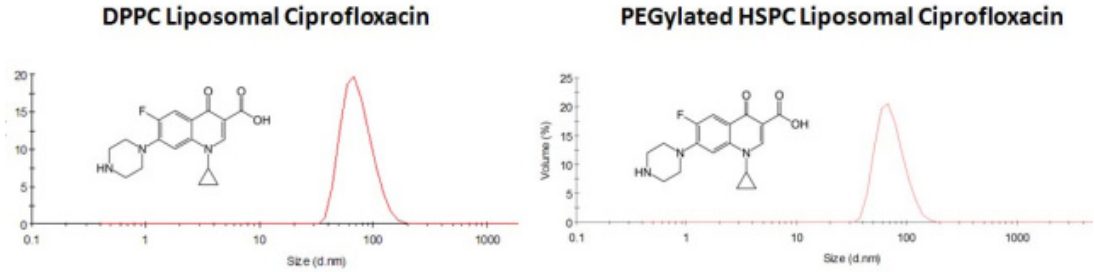


The **PEGylated Liposomal Doxorubicin - 2 mg (Catalog No. PHPC002DX)** contains 1 ml of liposomal doxorubicin at concentrations of 2 mg/ml doxorubicin and 16 mg/ml of lipids.

The **PEGylated Liposomal Doxorubicin - 20 mg (Catalog No. PHPC020DX)** contains 10 ml of liposomal doxorubicin at concentrations of 2 mg/ml doxorubicin and 16 mg/ml of lipids.

3.3.2 Liposomal ciprofloxacin

The PEGylated and non-PEGylated liposomal ciprofloxacin contains 1 mg/ml ciprofloxacin. The PEGylated liposome is composed of hydrogenated soy phosphocholine (HSPC), 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (DSPE-PEG2000) and cholesterol at a mass ratio of HSPC:DSPE:Cholesterol of 3:1:1. The Non-PEGylated liposome is composed of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and cholesterol at a mass ratio of DPPC:cheleterol of 1.9:1. The ciprofloxacin encapsulation percentage is more than 95 %. The average size of liposomes is about 80 nm with poly dispersity index (dpi) of 0.1.



The **DPPC Liposomal Ciprofloxacin- 2 mg (Catalog No. DPPC002CP)** contains 2 ml of liposomal ciprofloxacin at concentrations of 1 mg/ml ciprofloxacin and 23 mg/ml of lipids composed of HSPC and cholesterol.

The **PEGylated HSPC Liposomal Ciprofloxacin- 2 mg (Catalog No. PHPC002CP)** contains 2 ml of liposomal ciprofloxacin at concentrations of 1 mg/ml ciprofloxacin and 18 mg/ml of lipids composed of DSPE-PEG2000, HSPC and cholesterol.

3.4 Liposomal fluorescent dyes

Fluorescence dyes encapsulated in liposomes are used to track liposome distributions in biological systems.

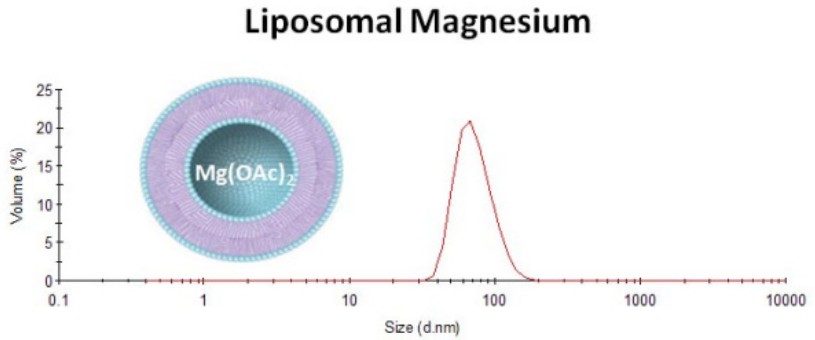
Catalog No	Fluorescence Dye	Excitation	Emission	Liposome composition
DPC001AO	Acridine orange	485 nm	535 nm	DPPC:Cholesterol = 1.9:1
DPC001RG	Rhodamine G	520 nm	580 nm	DPPC:Cholesterol = 1.9:1
DPC001FL	Fluorescein	485 nm	535 nm	DPPC:Cholesterol = 1.9:1
PHPC001AO	Acridine orange	485 nm	535 nm	HSPC:DSPE-PEG2000:Cholesteol = 3:1:1
PHPC001RB	Rhodamine B	520 nm	580 nm	HSPC:DSPE-PEG2000:Cholesteol = 3:1:1

The pH-sensitive fluorescence dyes such as Rhodamine delivered by liposomes into cells may be used to monitor pH changes in the cells. The liposomal fluorescence dyes contain 0.1 mM fluorescence dyes encapsulated in PEGylated or non-PEGylated liposomes. The total lipid concentration is 15 mg/ml. The PEGylated liposomes are composed of hydrogenated soy phosphocholine (HSPC), 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (DSPE-PEG2000) and cholesterol in a mass ratio of HSPC:DSPE-PEG2000:Cholesterol = 3:1:1. The non-PEGylated liposomes are composed of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and cholesterol in a mass ratio of 1.9:1. The fluorescence dye encapsulation percentage is more than 99 %. The average size of liposomes is about 80 nm with a poly dispersity index (dpi) of 0.1.

3.5 Liposomal ions

3.5.1 Liposomal magnesium

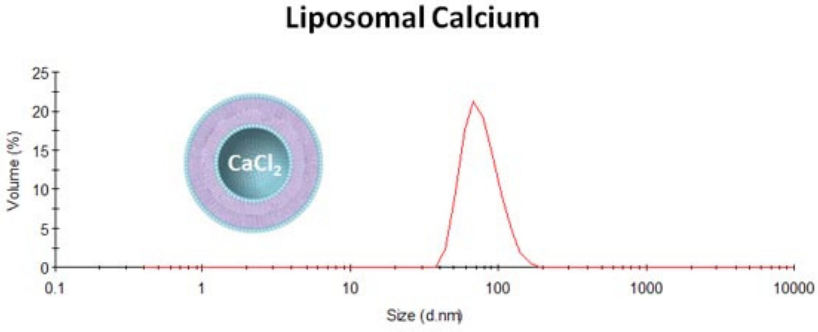
Liposomal magnesium is used to delivery of magnesium ions across the cell membranes. Magnesium acetate is encapsulated in liposomes composed of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and cholesterol in a mass ratio of 1.9:1 at total lipid concentration of 10 mg/ml. The magnesium concentration in the liposome is 150 mM. The magnesium encapsulation percentage is more than 99 %. The average size of liposomes is about 80 nm with poly dispersity index (dpi) of 0.1.



The **Liposomal Magnesium (Catalog No. DPC002MG)** includes 2 ml of 150 mM magnesium acetate encapsulated in liposomes.

3.5.2 Liposomal calcium

Liposomal calcium is used to delivery of calcium ions across the cell membranes. Calcium chloride is encapsulated in liposomes composed of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and cholesterol in a mass ratio of 1.9:1 at a total lipid concentration of 10 mg/ml. The calcium concentration in the liposome is 150 mM. The calcium encapsulation percentage is more than 99 %. The average size of liposomes is about 80 nm with poly dispersity index (dpi) of 0.1.



The **Liposomal Calcium (Catalog No. DPC002CA)** includes 2 ml of 150 mM calcium encapsulated in liposomes.

3.6 Liposomes and nanodiscs for membrane protein reconstitution

3.6.1 Liposomes for membrane protein reconstitution

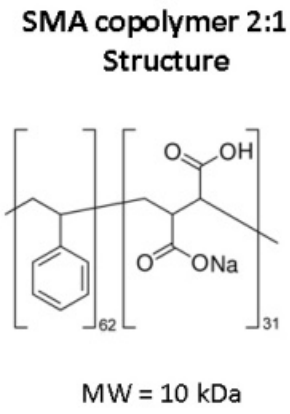
Reconstitution membrane proteins is often accomplished by incubation of purified membrane proteins with liposomes in the presence of detergent. The detergent is removed after the incubation by a hydrophobic column or extensive dialysis. The dialysis method can only be applied to detergents with a high CMC value. The following liposomes are currently available for membrane protein reconstitution:

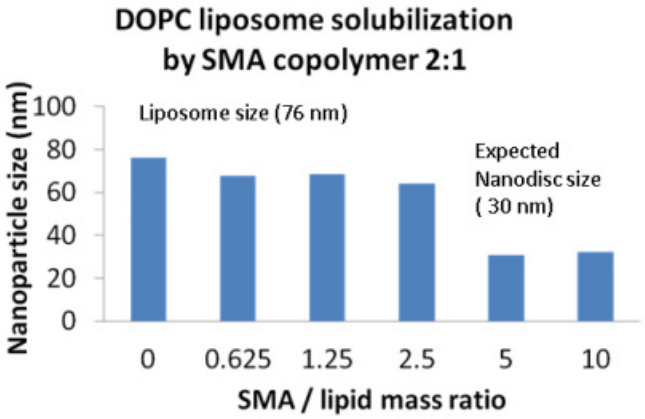
HSPC: Cholesterol: DSPG-PEG2000 liposome (Catalog No. HSCP010) contains 10 ml of 10 mg/ml liposome composed of HSPC: Cholesterol: DSPG-PEG2000 (m/m, 3:1:1).

DPPC: Cholesterol liposome (Catalog No. DPC010) contains 10 ml of 10 mg/ml liposome composed of DPPC: Cholesterol (m/m, 1.9:1).

3.6.2 SMA copolymer 2:1 for 30 nm nanodiscs

Styrene - maleic acid (SMA) copolymers are broadly used for function and structure studies of membrane proteins. The significant advantages of using SMA copolymers include (1) generating a detergent-free system and (2) forming bilayer nanodiscs with phospholipids. Application of SMA copolymers opens an avenue of membrane protein extraction from cell membranes and proteoliposomes in the absence of detergent. The extracted membrane proteins are stabilized in the nanodiscs that mimic the bilayer structure of lipids in nature. The membrane proteins in nanodiscs can be purified and employed in biochemical, biophysical and biological experiments. For example, the nanodiscs can be used for structure studies of membrane proteins by electronic microscopy (EM). Typically SMA copolymer 2:1 forms nanodiscs in a size about 30 nm while SMA copolymer 3:1 forms nanodiscs in a size about 10 nm. The ideal pH for nanodisc formation is between pH 7.0 – 8.0.



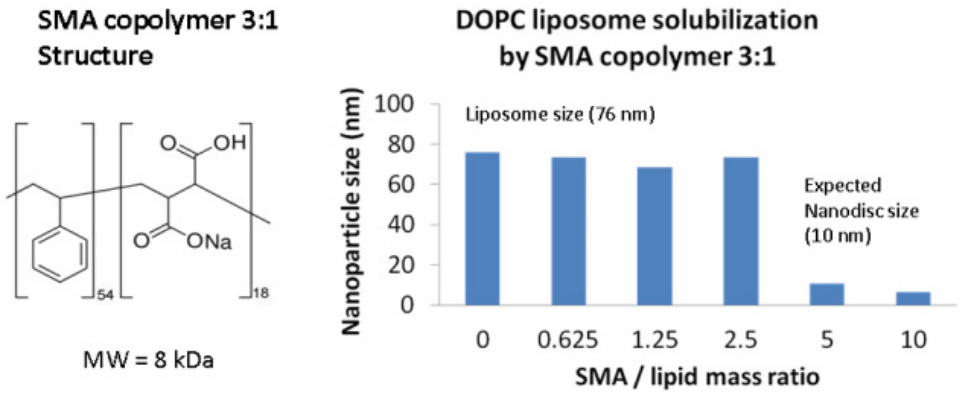


Styrene - Maleic Acid Copolymer 2:1 Free Acid is the free acid form of SMA Copolymer with a molar ratio of styrene to maleic acid of 2:1. The copolymer molecular weight is 10.1 kDa. The package size is 100 mg (Catalog No. SMA21-100MG) or 500 mg (Catalog No. SMA21-500MG).

Styrene - Maleic Acid Copolymer 2:1 Sodium Salt is the sodium salt form of SMA copolymer with a molar ratio of styrene to maleic acid of 2:1. The copolymer molecular weight is 10.4 kDa. The package size is 100 mg (Catalog No. SMA21S-100MG) or 500 mg (Catalog No. SMA21S-500MG).

3.6.3 SMA copolymer 3:1 for 10 nm nanodiscs

Styrene - maleic acid (SMA) copolymers are broadly used for function and structure studies of membrane proteins. The significant advantages of using SMA copolymers include (1) generating a detergent-free system and (2) forming bilayer nanodiscs with phospholipids. Application of SMA copolymers opens an avenue of membrane protein extraction from cell membranes and proteoliposomes in the absence of detergent. The extracted membrane proteins are stabilized in the nanodiscs that mimic the bilayer structure of lipids in nature. The membrane proteins in nanodiscs can be purified and employed in biochemical, biophysical and biological experiments. For example, the nanodiscs can be used for structure studies of membrane proteins by electronic microscopy (EM). Typically SMA copolymer 2:1 forms nanodiscs in a size about 30 nm while SMA copolymers 3:1 forms nanodiscs in a size about 10 nm. The ideal pH for nanodisc formation is between pH 7.0 – 8.0.



Styrene - Maleic Acid Copolymer 3:1 Free Acid is the free acid form of SMA Copolymer with a molar ratio of styrene to maleic acid of 3:1. The copolymer molecular weight is 7.8 kDa. The package size is 100 mg (Catalog No. SMA31-100MG) or 500 mg (Catalog No. SMA31-500MG).

Styrene - Maleic Acid Copolymer 3:1 Sodium Salt is the sodium salt form of SMA copolymer with a molar ratio of styrene to maleic acid of 3:1. The copolymer molecular weight is 8.6 kDa. The package size is 100 mg (Catalog No. SMA31S-100MG) or 500 mg (Catalog No. SMA31S-500MG).

3.6.4 Nanodiscs for membrane protein reconstitution

Nanodiscs composed of lipids and SMA copolymer provide the planner bilayer structure for membrane protein reconstitution.

10 nm DOPC Nanodisc (Catalog No. ND-OCGS-10) contains 2 ml of 5 mg/ml lipids composed of DOPC:Cholesterol: DSPE-PEG2000 and 40 mg/ml SMA copolymer 3:1 in 0.1 M TrisHCl, pH 7.6.

30 nm DOPC Nanodisc (Catalog No. ND-OCGS-30) contains 2 ml of 5 mg/ml lipids composed of DOPC:Cholesterol: DSPE-PEG2000 and 40 mg/ml SMA copolymer 2:1 in 0.1 M TrisHCl, pH 7.6.

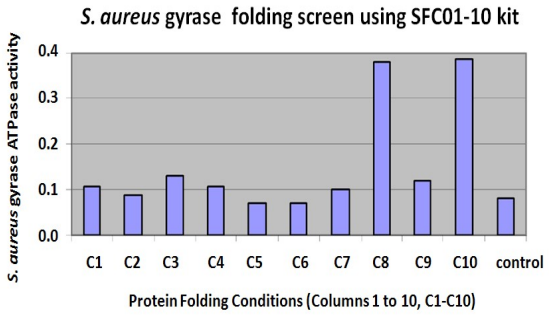
Chapter 4
Protein folding, extraction, reconstitution and stability

4.1 Preparation of active proteins from inclusion bodies

4.1.1 Protein folding columns for folding non-membrane proteins

ProFoldin protein folding columns are designed to produce active proteins from guanidine hydrochloride or urea-solubilized inclusion bodies or protein aggregates formed during protein expression, purification or storage. Different proteins are folded under different conditions. The Spin-column Protein Folding Screen Kit includes 10 spin-columns that represent 10 optimized and diversified protein folding conditions including conditions allowing disulfide bond formation and reducing conditions. The folded protein samples from the screen kit are used for SDS-PAGE and activity tests. Based on the test results, the optimal condition (the column number) is selected for preparative folding.

Spin-Column Protein Folding Screen Kit
Small-Scale Preparative Protein Folding Column Set



Spin-Column Protein Folding Screen Kit (Catalog No. SFC01-10) contains 0.16 ml Solution A, 0.6 ml Solution B, 10 spin columns.

Preparative protein folding column sets

The Preparative Protein Folding Column Sets are used for preparative protein folding after the folding condition has been identified by the Spin-column Protein Folding Screen Kit (Catalog No SFC01-10). The column number represents the specific folding condition.

Small-Scale Preparative Protein Folding Column Set (Catalog No. SFC01 to SFC10) contains 0.16 ml Solution A, 0.6 ml Solution B, 10 spin columns. Each column set includes 10 identical preparative protein folding columns and reagents for folding 0.5 to 1 mg of denatured proteins.

Large-Scale Preparative Protein Folding Column Set (Catalog No. PFC01) contains 5.4 ml Solution A, 8.4 ml Solution B, 14.4 ml Solution C, 4 preparative columns. The column set includes 4 identical preparative protein folding columns and reagents for folding 10 to 20 mg of guanidine hydrochloride or urea-solubilized protein.

4.1.2 Protein folding solutions for folding non-membrane proteins

96-Well Protein Folding Plate (Catalog No. PFS096)

Kit components: One 96-well plate with 96 protein folding solutions, 0.5 ml in each well of the mother plate; 1.4 ml of Inclusion Body Solubilizer; 4 ml of Neutralizer. Each experiment uses 0.1 ml of the solutions from the mother plate. Each mother plate contains 0.5 ml of solutions in each well and can be used for multiple experiments of folding various proteins.

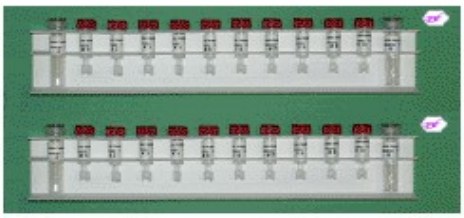
The 96-Well Protein Folding Plate provides 96 diversified conditions for protein folding screens. The 96 folding conditions include various pHs, salt concentrations and additives. Each experiment uses 0.1 ml of the solutions from the mother plate. Each mother plate contains 0.5 ml of solutions in each well and can be used for multiple experiments of folding various proteins. About 20 micrograms of guanidine hydrochloride-solubilized proteins from inclusion bodies are used for each condition. Once the active protein is identified, preparative folding solutions for particular conditions are available for preparative scale folding.

Protein Folding Solution Kit (Catalog No. PFS well#) contains 5 ml inclusion body solubilizer, 40 ml 2 x PFS, 4 ml neutralizer.

4.1.3 Protein folding columns for folding membrane proteins

Spin-column Membrane Protein Folding Screen Kit (Catalog No. MFC01-20) contains 0.16 ml Reagent A, 0.16 ml Reagent B, 1.1 ml Reagent C, 20 spin columns.

The Spin-column Membrane Protein Folding Kit is designed for folding membrane proteins from inclusion bodies produced in the cells during protein over-expression. The kit includes 20 protein folding spin columns representing 20 different conditions with various detergents and



Spin-Column Membrane Protein Folding Screen Kit

lipids that form micelles or bicelles. The micellar or bicellar environments facilitate folding receptors, ion channels and other membrane proteins. The folded protein samples from the screen kit are used for SDS-PAGE and activity tests. Based on the test results, the optimal condition (the column number) is selected for preparative folding. The Large-scale Membrane Preparative Protein Folding Column Set (MFC01 to MFC20) is for folding of 10 to 20 mg of denatured membrane proteins.

Preparative Membrane Protein Folding Column Set (Catalog No. MFC01 to MFC20) contains 5.4 ml Reagent A or Reagent B, 8.4 ml Reagent C, 14.4 ml Solution S, and 20 spin columns.

The Preparative Membrane Protein Folding Column Sets are used for preparative membrane protein folding after the folding condition has been identified by the Spin-column Membrane Protein Folding Screen Kit (Catalog # MFC01-20). The column number represents the specific folding condition. Each Column Set includes 4 identical preparative protein folding columns and reagents for folding about 5 mg of urea-solubilized inclusion body proteins.

4.1.4 Protein folding solutions for folding membrane proteins

Dilution Membrane Protein Folding Screen Kit (Catalog No. MPS01-20) contains 0.1 ml Reagent A, 0.1 ml Reagent B, 1.1 ml Reagent C, 0.35 ml Solution S1 - S10.

ProFoldin Dilution Membrane Protein Folding Screen Kit (catalog # MPS01-20) provides 20 optimized conditions for screens of membrane protein folding conditions. About 70 μg of urea-solubilized proteins from inclusion bodies are used for each condition. Once the folding conditions are identified, preparative folding kits are available for preparative scale folding. Each preparative folding kit is for folding 5 mg of urea-solubilized proteins. The condition number is identical to the Solution S number in the kit.

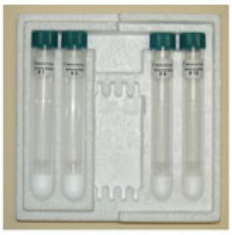
Dilution Preparative Membrane Protein Folding Kit (Catalog No. MPP01 to MPP20) contains 0.4 ml Reagent A, 0.4 ml Reagent B, 7.2 ml Reagent C, 24.9 ml Solution S1 to S10.

4.2 Extraction of membrane proteins from cell membranes

ProFoldin's Membrane Protein Extract Solutions provide optimal conditions for efficient extraction and stabilization of membrane proteins from the cell membranes by using a variety of non-denaturing detergent and synthetic lipid analogs including alkyl saccharides (MPE01, MPE02), acyl-N-methylglucamide (MPE 04), bile acid salt (MPE 06), alkylaminoxide (MPE 07), alkylpolyethylenes (MPE 03, MPE 05, MPE 11), zwitterionic detergents (MPE 08, MPE 09, MPE 12), and synthetic phosphocholine derivative (MPE 10). The Membrane Protein Extraction Kit (MPE01-12S) includes 0.5 ml of 12 solutions for screening and micro-scale preparative extraction. The Membrane Protein Extraction Kit Plus (MPE01-12P) includes 5 ml of 12 solutions for screening and middle-scale preparative membrane protein extraction (10 – 15 mg of total membrane protein). The Membrane Protein Extraction Solution (MPE01 to MPE12) provides 40 ml of a specific solution for large-scale preparative extraction (80 - 120 mg of total membrane protein).

Membrane Protein Extraction Kit
(Catalog number: MPE01-12S)

Membrane Protein Extraction Kit Plus
(Catalog number: MPE01-12P)



Membrane Protein Extraction Solution
(Catalog numbers: MPE01 to MPE12)

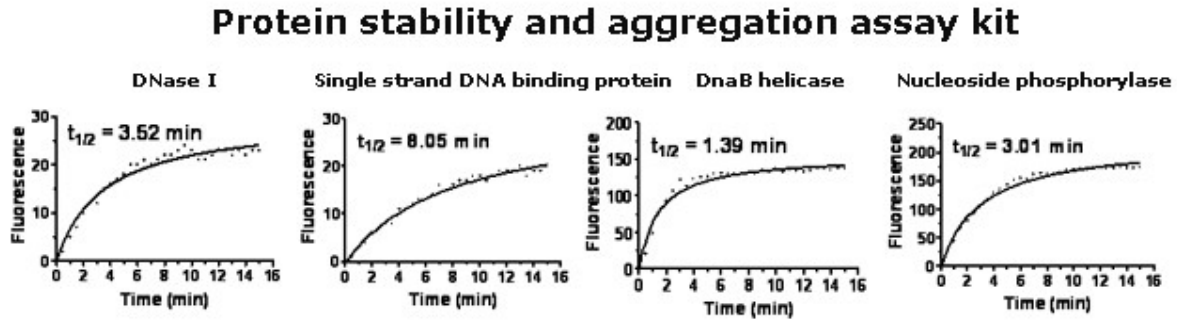
Membrane Protein Extraction Kit (Catalog No. MPE01-12S) contains 0.5 ml MPE1 - MPE12.
Membrane Protein Extraction Kit Plus (Catalog No. MPE01-12P) contains 5 ml MPE1 - MPE12.
Membrane Protein Extraction Solution (Catalog No. MPE01 to MPE12) contains 40 ml MPE.

4.3 Nanodiscs for membrane protein reconstitution

For information of Nanodiscs for membrane protein reconstitution, please see **Section 3.6** "**Liposomes and nanodiscs for membrane protein reconstitution"**

4.4 Detection of protein aggregation and thermal stability

The **Protein Stability and Aggregation Assay Kit** is for evaluation of protein thermal stability and analysis of protein unfolding aggregation. The assay is based on a fluorescence dye binding to the hydrophobic surfaces of the proteins and generates fluorescence at 610 nm. Since the unfolded (denatured) proteins have more hydrophobic surfaces than the native ones, the unfolded protein generates a higher fluorescence intensity than the native protein does at the same protein concentration in the same buffer. By incubation of the native protein at a raised temperature, a thermal unfolding curve can be observed and the half life time ($t_{1/2}$) of the protein is calculated. The half life time value can be used to evaluate protein stability such as comparison between the wild-type and mutant proteins. The thermal unfolding curve can also be used to analyze the protein unfolding aggregation state of protein samples.



Protein unfolding reactions at 55°C are measured by the Protein stability and aggregation assay kit (Catalog number PSA200K). The protein concentrations are 0.2 mg/ml DNase I, 0.25 mg/ml single DNA binding protein, 0.18 mg/ml DnaB helicase and 0.9 mg/ml nucleoside phosphorylase.

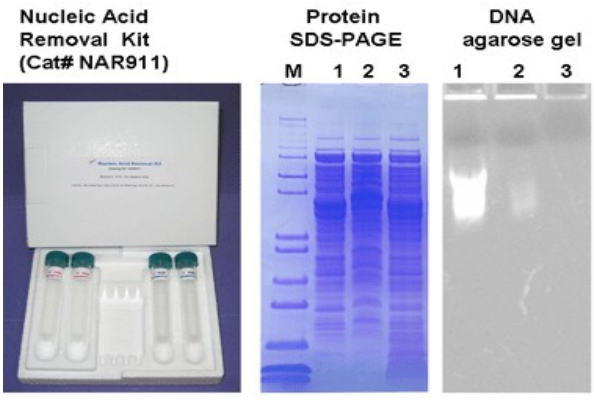
Protein stability and aggregation assay kit (Catalog No PSA200K) contains the 1000 x PSA dye for 200 assays with the assay volume of 0.3 ml using 96-well plates or cuvettes.

Chapter 5
Purification kits

Separation of certain molecules from others are often needed in analytical or production work. ProFoldin provides tools for removal nucleic acids, proteins, phosphates or small molecules form macromolecules.

5.1 Remove nucleic acids

The Protein and DNA Removal Columns are designed to separate small molecules and liposomes from proteins, DNA and RNAs. The columns can be used for separation of free drugs and liposome-encapsulated drugs from protein-bound or DNA-bound drugs, free ligands from receptor-bound ligands. They can also be used for preparation of HPLC samples by removing the DNA or proteins from biological samples. The column resin is highly charged and binds DNA, RNA and proteins. The binding between the biological molecules and the column resin is mainly charge-charge interactions. Hydrophobic interactions may also contribute to the binding. The proteins, nucleic acids and protein-bound drug stay on the column while the small polar molecules or liposomes are in the elute. Small but very hydrophobic molecules may also bind to the column. The binding capacity of the spin columns is more than 0.10 mg of protein or 0.02 mg of DNA per column. The binding capacity of the preparative columns is more than 1.20 mg of protein or 0.24 mg of DNA per column.

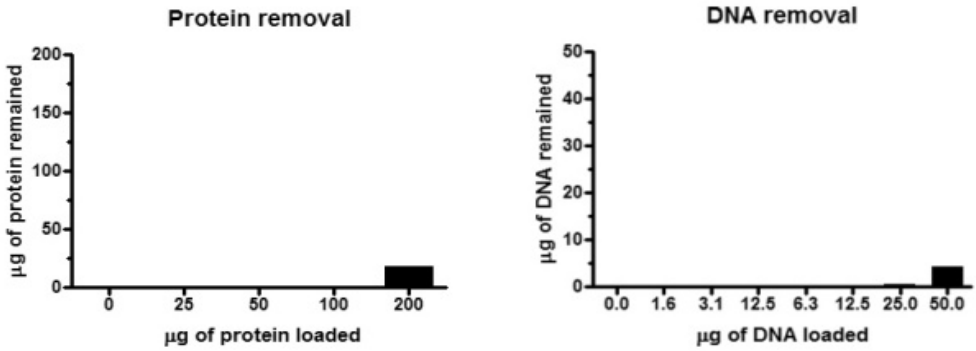


Nucleic Acid Removal Kit (Catalog No. NAR911)

Kit components: 22 ml NAR reagent 1, 22 ml NAR reagent 2.

5.2 Remove proteins and nucleic acids

The Protein and DNA Removal Columns are designed to separate small molecules and liposomes from proteins, DNA and RNAs. The columns can be used for separation of free drugs and liposome-encapsulated drugs from protein-bound or DNA-bound drugs, free ligands from receptor-bound ligands. They can also be used for preparation of HPLC samples by removing the DNA or proteins from biological samples. The column resin is highly charged and binds DNA, RNA and proteins. The binding between the biological molecules and the column resin is mainly charge-charge interactions. Hydrophobic interactions may also contribute to the binding. The proteins, nucleic acids and protein-bound drug stay on the column while the small polar molecules or liposomes are in the elute. Small but very hydrophobic molecules may also bind to the column. The binding capacity of the spin columns is more than 0.10 mg of protein or 0.02 mg of DNA per column. The binding capacity of the preparative columns is more than 1.20 mg of protein or 0.24 mg of DNA per column.



Protein and DNA Removal Spin-columns (Catalog No. PNR020)

Kit components: 20 spin columns.

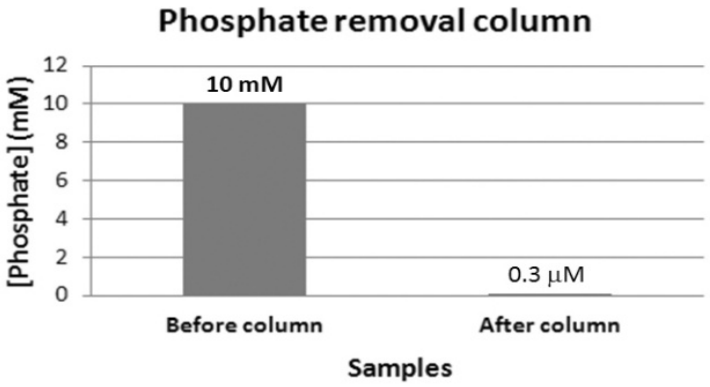
Preparative Protein and DNA Removal Columns (Catalog No. PNR04P)

Kit components: 4 PD-10 columns.

5.3 Remove phosphate

ProFoldin phosphate removal columns are designed to remove phosphate from a buffer solution. For example, the phosphate concentration can be reduced from 10 mM to 0.001 mM or below. The principle of phosphate removal is based on interactions between phosphate and

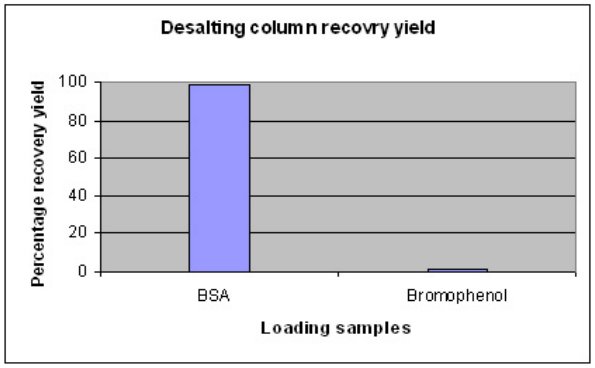
the column resin. The phosphate stays on the column. The phosphate binding capacity of the resin is 20 μmole per milliliter of the bed volume. Other buffer components including regular buffer salts and non-charged molecules such as sugar and glycerol do not bind to the resin and therefore stay in the sample solution. Some organic phosphates such as ATP also bind to the resin but not as strongly as inorganic phosphate. Metal chelators such as EDTA interfere the phosphate-resin binding.



Micro Phosphate Removal Column Set (Catalog No. MPR020)
Kit components: 20 spin columns.

5.4 Remove salt or buffer exchange

ProFoldin desalting columns are designed to remove small molecules like salts, free enzyme substrates or ligands from a protein or DNA solution. After desalting, the protein or DNA sample is in a low-salt buffer composed of 10 mM Tris-HCl, pH 7.5. The columns can also be



used for buffer exchange of protein or DNA samples to a desired buffer which is used to pre-equilibrate the column. The principle of desalting is size-exclusion chromatography with a

molecular cut-off of 5 kDa. The residual salt concentration in the desalted solution is less than 2 % of the original salt concentration. The protein recovery yield is 98 % or higher

Micro Desalting Spin Columns Set (Catalog No. MDC050)

Kit components: 50 spin columns.

Preparative Desalting Column Set (Catalog No. PDC020)

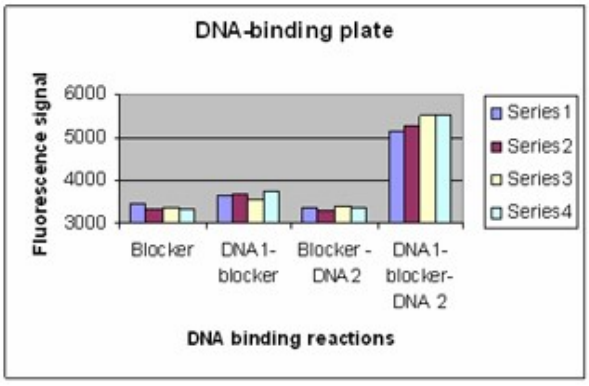
Kit components: 20 PD-10 columns.

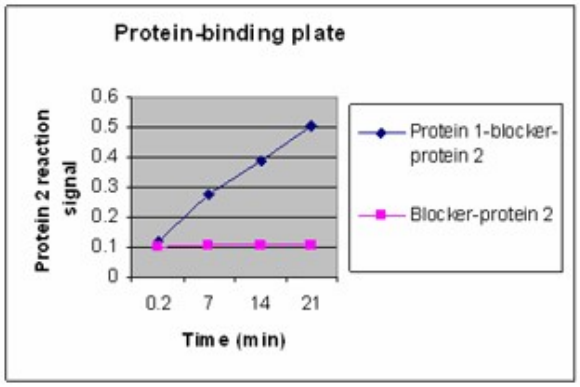
Chapter 6
Molecular binding assays

ProFoldin offers highly charged 96-well plates for DNA or protein binding. The biological molecule-bound plates can be used to study the interactions between the bound molecule and other molecules. They can be used for ELISA, studies of interactions between nucleic acids or/and proteins.

6.1 DNA and protein binding plates

Specific interactions between biomolecules are studied by using highly charged 96-well plates. For instance, negatively charged DNA, RNA or proteins (the first biomolecule) are bound to the highly positively charged plates. After the plates are blocked with the Nucleic Acid Binding Blocker or BSA, a second biomolecule is added. The second biomolecule will also bind to the plate if it interacts with the first biomolecule. The second molecule can be detected by enzymatic assays if it is an enzyme, fluorescence assay if it is DNA or RNA, or ELISA if its antibody is available. If the second molecule is fluorescence or isotope labeled, the labeled signals can be detected. We provide highly charged 96-well black or transparent polystyrene plates for various applications.





DNA-binding plates:

The DNA-binding plates are coated with cations that interact with nucleic acids which are anions. Since the binding is based on charge-charge interactions, the plates may also be used for RNA binding. If the bound DNA is a single-strand DNA, the bound DNA can specifically recognize its complementary strand by G-C and A-T base pairing. Therefore, the 96-well DNA-

binding plates can be potentially used for studies of nucleic acid – nucleic acid interactions or nucleic acid – protein interactions.

96-well DNA–binding Plates (black) (Catalog No: NBP96B) includes 4 plates. The black background of the plate provides a minimum noise level for fluorescence detection of the bound molecules.

96-well DNA–binding Plates (transparent) (Catalog No: NBP96T) includes 4 plates. The transparent background of the plate provides an option of detection of the bound molecules based on light absorbance.

Nucleic Acid Binding Blocker (Catalog No: NBP96N) includes 1 ml of 200 x concentrated stock solution. The Nucleic Acid Binding Blocker is used to block non-specific interactions between nucleic acids and positively charged surfaces. In a hetero-phase nucleic acid - nucleic acid interaction, after the first nucleic acid binds to the positively charged solid phase, the solid surface was blocked by the blocker so that only the nucleic acid that recognizes the first nucleic acid will bind to the surface when the surface is exposed to various nucleic acids.

Protein-binding plates

The 96-well Protein-binding Plates can be used to study specific protein-protein, protein-nucleic acid or protein-ligand interactions.

96-well Anionic Protein-binding Plates (black) (Catalog No: APP96B) includes 4 plates. The Anionic Protein-binding Plates bind negatively charged proteins. The black background of the plate provides a minimum noise level for fluorescence detection of the bound molecules.

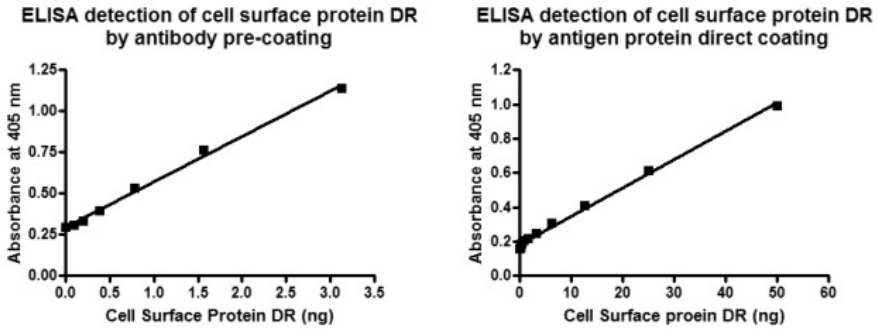
96-well Anionic Protein-binding Plates (transparent) (Catalog No: APP96T) includes 4 plates. The Anionic Protein-binding Plates bind negatively charged proteins. The transparent background of the plate provides an option of detection of the bound molecules based on light absorbance.

96-well Cationic Protein-binding Plates (black) (Catalog No: CPP96B) includes 4 plates. The Cationic Protein-binding Plates bind positively charged proteins. The black background of the plate provides a minimum noise level for fluorescence detection of the bound molecules.

96-well Cationic Protein-binding Plates (transparent) (Catalog No: CPP96T) includes 4 plates. The Cationic Protein-binding Plates bind positively charged proteins. The transparent background of the plate provides an option of detection of the bound molecules based on light absorbance.

6.2 96-well plates for ELISA

The 96-well Super High Binding ELISA Plates are designed for strong signals of ELISA. The ELISA sensitivity is dramatically enhanced by strong protein binding on the plates. In a direct binding mode, the antigen is coated onto the plate first. After blocking, the antibody against the antigen is bound to the antigen and the bound antibody is detected by an enzyme-linked second antibody. Alternatively, if two antibodies (A and B) against the antigen are available, antibody A is coated onto the plate first. After blocking, the antigen is bound to the coated antibody A. Then antibody B is bound to the antigen. Finally the bound antibody B is detected by the enzyme-linked antibody.



The **96-well Super High Binding ELISA Plates – Transparent (Catalog No. ELISA96T)** are for absorbance detection of the reaction catalyzed by the enzyme-linked antibody. Each plate set includes 4 plates.

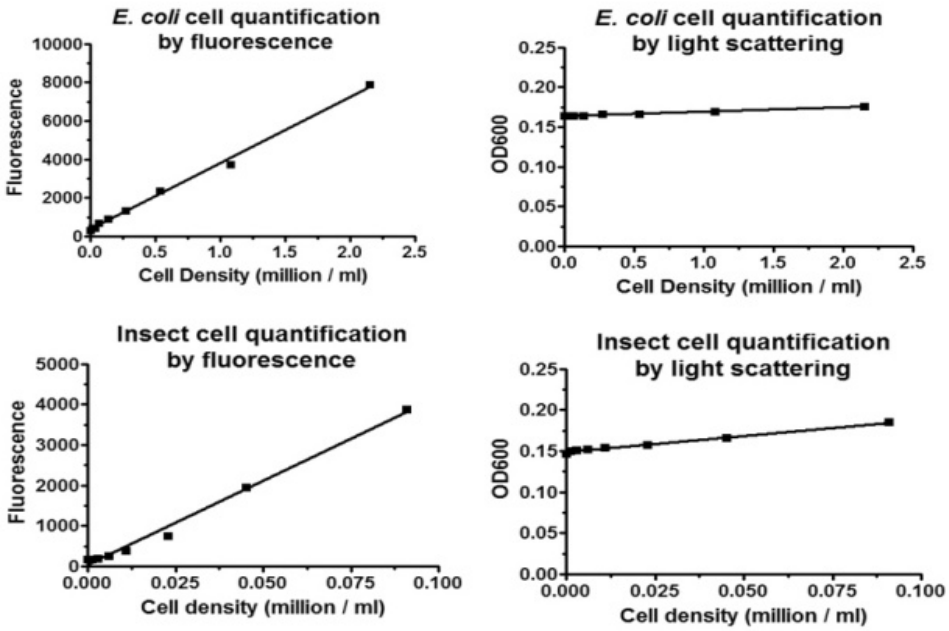
The **96-well Super High Binding ELISA Plates – Black (Catalog No. ELISA96B)** are for fluorescence detection. Each plate set includes 4 plates.

Chapter 7

Cell density and tissue staining

7.1 Sensitive cell density assays

The Cell Assay Dye detects cells by fluorescence emission at 535 nm with excitation at 485 nm. It can be used for quantification of cells with two unique features: (1) high sensitivity and (2) high throughput. For example, a cell density of *E. coli* cells with 0.01 OD_{600} gives a fluorescence signal to background ratio of 25. It can measure cells with cell density beyond OD_{600} detection range (OD_{600} below 0.001). The assay is in a 96-well plate format and can be used for high throughput detection of bacterial and other cells.



Cell Separation Filter Plate (Catalog No. CSF1)

Kit components: 4 CFS plates.

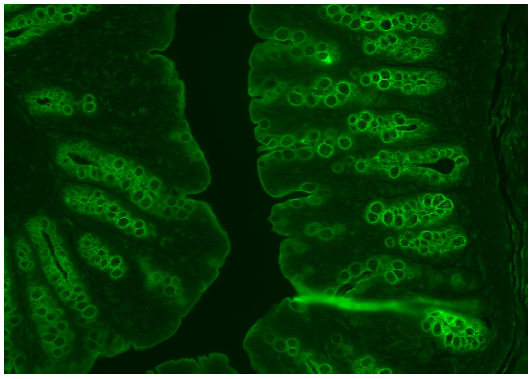
Cell Density Assay Dye (Catalog No. CP1000)

Kit components: 0.5 ml 100x Dye CP1.

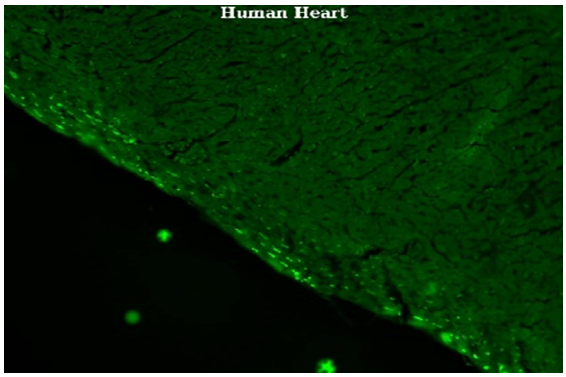
7.2 Tissue / cell / particle fluorescence staining

Highly positively charged fluorescence dyes are available for tight binding of negatively charged cells, tissues or particles. The selective and tight binding of the highly positively charged fluorescence dyes on the negatively charged surfaces allows extensive washing and low background of the staining effect. These dyes can be used for visualization of specific cells, tissues or particles, for example, applications in histology.

Rat colon



Human heart



Tissue staining fluorescence dye (Catalog No. CS1000)

Kit components: 0.1 ml of 3000 x stock solution.

Chapter 8
Publications of product applications

ProFoldin's products are broadly employed by pharmaceutical and biotech companies whose publications are often limited by companies' publication policies. Most of the following publications are by the academic labs.

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6. Pelletier D.M, Hemoglobin binding protein from *Actinobacillus pleuropneumoniae*: a novel method for extraction and isolation. McGill University, Montreal Q.C. Graduate thesis, (2007). [Membrane protein folding columns, MFC01-20]

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14. Siegmund V. et al, Screening mutant libraries of T7 RNA polymerase for candidates with increased acceptance of 2'-modified nucleotides, Chem. Commun., 48, 9870-9872 (2012). [T7 RNA polymerase assay kit, T7RPA100K]

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16. Copeland D.L., Production of Recombinant Carp Leptin And its Effects on Lipid Metabolism in the Common Carp (Cyprinus Carpio), Thesis, The University of Akron (2012). [Protein folding columns, SFC01-10]

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