BQC

REDOX TECHNOLOGIES

KB03003

Bradford Protein Quantification Assay Kit

2000/4000 tests (96 well plate, high concentrations)
3333/6666 test (96 well plate, low concentrations)
500/1000 tests (test tube)

Table of contents

1. General information

PRECAUTIONS

Please read this manual carefully before beginning the assay.

This product is designed for **research use only**. It is not approved for human or animal use or clinical diagnosis. All chemicals should be handled with care and in accordance with laboratory safety practices. It is recommended to use basic Personal Protective Equipment.

Do not use after the expiration date stated on the packaging.

Do not mix or substitute reagents or materials from other kit batches or vendors.

For the **material safety data sheet** (MSDS) please contact us at info@bioquochem.com

TECHNICAL RECOMMENDATIONS

Store reagents as indicated in **Materials and storage** section.

Be sure to keep the bottle capped when not in use.

Let the components reach room temperature (RT) before use.

Immediately before use, gently invert and rotate reagent bottles several times to mix the contents thoroughly.

Avoid foaming or bubbles when mixing or reconstituting components.

Avoid cross contamination of samples or reagents by changing pipette tips between sample, standard and reagent additions.

Be sure to use the optimal microplate for the assay. Flat bottom transparent microplates for UV/VIS applications, and black microplates for fluorescence measurements.

2. Technical specifications

Available sizes

Test tube format: 500/1000 tests

Microplate format:

- 2000/4000 tests (high concentrations)
- 3333/6666 test (low concentrations)

Required sample volume

Test tube format: 1 mL/test

Microplate format:

- 5 µL/test (high concentrations)
- 150 µL/test (low concentrations)

Compatible samples

Biological fluids, food, beverages, and other samples

Type of detection

Colorimetric (595 nm)

3. Materials and storage

MATERIALS SUPPLIED

Item	No. Tests	Units	Storage
Reagent A	2000	1	4 °C
	4000	2	
Protein Standard	2000	1	4 °C
	4000	2	
Transparent 96-Well Microplate	2000	1	RT
	4000	2	

MATERIALS NEEDED BUT NOT SUPPLIED

- Double distilled water (ddH_2O) as Milli-Q Ultrapure Water.
- Labware materials (micropipettes, tubes, stirring/mixing equipment).
- Colorimetric microplate reader – equipped with filter for OD 595 nm.

STORAGE CONDITIONS

On receipt, store kit components as indicated above. Under these conditions, the reagents are stable in the original packaging until the expiration date indicated on the outside of the box. After reconstitution, protein standard solution should be stored at -20 °C. Prepare a fresh set of standards for every use.

4. Introduction

The Bradford Protein Assay is a biochemical assay for determining the total level of protein in a solution, first described by Marion M. Bradford in 1976.

Proteins are biopolymeric structures composed of amino acids that play many critical roles in the body. Protein is also a vital part of the human diet. Protein quantification assays are therefore fundamental to biological research, clinical diagnosis or food industry.

The BCA assay has many advantages over the alternatives (e.g. Lowry, Bradford) including compatibility with most salts, solvents, buffers, thiols, reducing substances, and metal chelating agents encountered in protein samples.

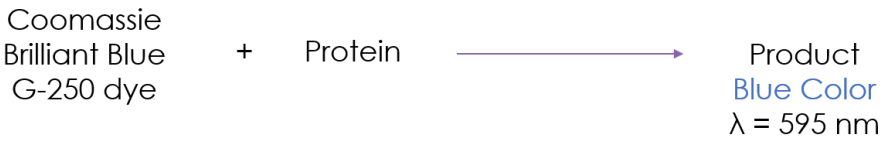
The main disadvantage of this assay is its incompatibility with surfactants at concentrations routinely used to solubilize membrane proteins.

BQC Bradford Protein Quantification Assay Kit is a simple and fast test for the quantification of proteins in a wide variety of samples.

5. Assay principle

BQC Bradford Protein Quantification Assay Kit is based on the Bradford assay. The Bradford protein assay is a dye-binding assay based on the differential color change of a dye in response to protein concentration. In an acidic media, proteins bind to Coomassie Brilliant Blue G-250 dye producing a shift of the dye maximum absorbance wavelength from 465 to 595 nm.

The amount of the dye-protein complex formed is directly related to a protein concentration and can be spectrophotometrically estimated by measuring the absorbance at 595 nm. The protein concentration is a sample is determined from a calibration curve using bovine serum albumin (BSA) as standard.



Principle of the Bradford Protein Assay Kit

6. Assay preparation

REAGENT PREPARATION

All assay reagents not listed below are ready to use as supplied. Allow the reagents to reach room temperature before use.

Protein Standard Solution (Bovine Serum Albumin, BSA): Add 3 mL of ddH_2O to the Standard vial. Mix carefully to avoid foaming.

> **NOTE: Aliquot and store at -20 °C the Standard Solution** for long term use.

Use this **Standard solution** to prepare the **standard curve** for the **microplate assay (high concentration, 50-1500 µg/mL)**

Dilute the **Standard solution** 1:100 with ddH_2O (e.g. 10 µL of Standard Solution with 990 µL of ddH_2O). Use this **1:100 diluted Standard solution** to prepare the standard curve for the **test tube** assay and for the **microplate assay (low concentration,1-30 µg/mL)**

STANDARD CALIBRATION

Microplate format (high concentration, 50-1500 µg/mL)

Prepare BSA standards for the calibration curve from the Protein Standard Solution according to the following Table. Prepare the standards immediately prior to each assay. Mix carefully to avoid foaming.

Standard	Standard solution (µL)	*Diluent (µL)	Protein (µg/mL)
Std 1 (Reagent Blank)	0	200	**0**
Std 2	1	199	**50**
Std 3	2	198	**100**
Std 4	4	196	**200**
Std 5	8	192	**400**
Std 6	12	188	**600**
Std 7	20	180	**1000**
Std 8	30	170	**1500**

*Use as diluent the buffer used in the samples

Microplate format (low concentration, 1-30 µg/mL)

Prepare BSA standards for the calibration curve from the 1:100 diluted Standard solution according to the following Table. Prepare the standards immediately prior to each assay. Mix carefully to avoid foaming.

Standard	Standard solution 1:100 diluted (µL)	*Diluent (µL)	Protein (µg/mL)
Std 1 (Reagent Blank)	0	600	**0**
Std 2	6	594	**1**
Std 3	30	570	**5**
Std 4	60	540	**10**
Std 5	120	480	**20**
Std 6	180	420	**30**

*Use as diluent the buffer used in the samples

Test tube format

Prepare BSA standards for the calibration curve from the 1:100 diluted Standard solution according to the following Table. Prepare the standards immediately prior to each assay. Mix carefully to avoid foaming. .

Standard	Standard solution 1:100 diluted (mL)	*Diluent (mL)	Protein (µg/mL)
Std 1 (Reagent Blank)	0	3.00	**0**
Std 2	0.03	2.97	**1**
Std 3	0.15	2.85	**5**
Std 4	0.30	2.70	**10**
Std 5	0.60	2.40	**20**
Std 6	0.90	2.10	**30**

*Use as diluent the buffer used in the samples

PLATE SET UP

BQC recommends running the standards and samples at least in duplicate (triplicate recommended). There is no specific pattern for using the wells on the plate. A proposed layout of standards (Std) and samples (S) to be measured in duplicate is shown below.

NOTE: If sample blanks are included in the assay, it is necessary to reserve some wells of the plate for these blanks

	1	2	3	4	5	6	7	8	9	10	11	12
A	Std 1	Std 1	S3	S3	S11	S11	S19	S19	S27	S27	S35	S35
B	Std 2	Std 2	S4	S4	S12	S12	S20	S20	S28	S28	S36	S36
C	Std 3	Std 3	S5	S5	S13	S13	S21	S21	S29	S29	S37	S37
D	Std 4	Std 4	S6	S6	S14	S14	S22	S22	S30	S30	S38	S38
E	Std 5	Std 5	S7	S7	S15	S15	S23	S23	S31	S31	S39	S39
F	Std 6	Std 6	S8	S8	S16	S16	S24	S24	S32	S32	S40	S40
G	S1	S1	S9	S9	S17	S17	S25	S25	S33	S33	S41	S41
H	S2	S2	S10	S10	S18	S18	S26	S26	S34	S34	S42	S42

Example of plate layout for the Bradford Protein Quantification Assay Kit

7. Sample preparation

The following sample preparation protocols are intended as a guide only. The optimal conditions for sample preparation must be determined by the end user. It is recommended to use fresh samples. If it is not possible, aliquot and store samples appropriately with minimal freeze/thawing.

Bradford Protein Quantification Assay Kit can be used to determine proteins in a wide variety of samples like biological fluids, food, and beverages.

Reagents and materials required for sample preparation are not supplied with the kit. Before doing sample preparation, consider the volume of sample required per test; see **Technical specifications** section.

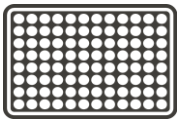
Make sure that interfering substances present in the sample do not give a significant background. Run proper blanks as necessary (e.g. sample blank should be always evaluated when working with highly colored samples). It is recommended to assay different sample dilutions to ensure the values fall within the standard curve.

8. Assay protocol

Prepare and mix all reagents thoroughly before use. Each standard, sample or blank should be assayed at least in duplicate.

- **CAUTION:** Mix the **Bradford Reagent** solution immediately before use by gently inverting the bottle a few times

Microplate Bradford Protein Quantification Kit (Working range: 50-1500 µg/mL)

1  Set up the plate design

2  Add **5 µL** of **standard** or **sample** in each well

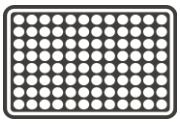
3  Add **250 µL** of **Bradford Reagent** in each well and mix by pipetting

4  **Incubate** the microplate for **at least 5 minutes** at **RT**

- The incubation step should not be longer than one hour

5  Read the **absorbance** of all wells at **595 nm** in end point mode at **RT**

Microplate Bradford Protein Quantification Kit (Working range: 1-30 µg/mL)

1  Set up the plate design

2  Add **150 µL** of **standard** or **sample** in each well

3  Add **150 µL** of **Bradford Reagent** in each well and mix by pipetting

4  **Incubate** the microplate for **at least 5 minutes** at **RT**

- The incubation step should not be longer than one hour

5  Read the **absorbance** of all wells at **595 nm** in end point mode at **RT**

Test tube Bradford Protein Quantification Kit (2 mL) (Working range: 1-30 µg/mL)

1  Pipette **1 mL** of **standard** or **sample** into 2 mL clean microcentrifuge tubes (not included)

2  Add **1 mL** of **Bradford Reagent** to each tube and mix well

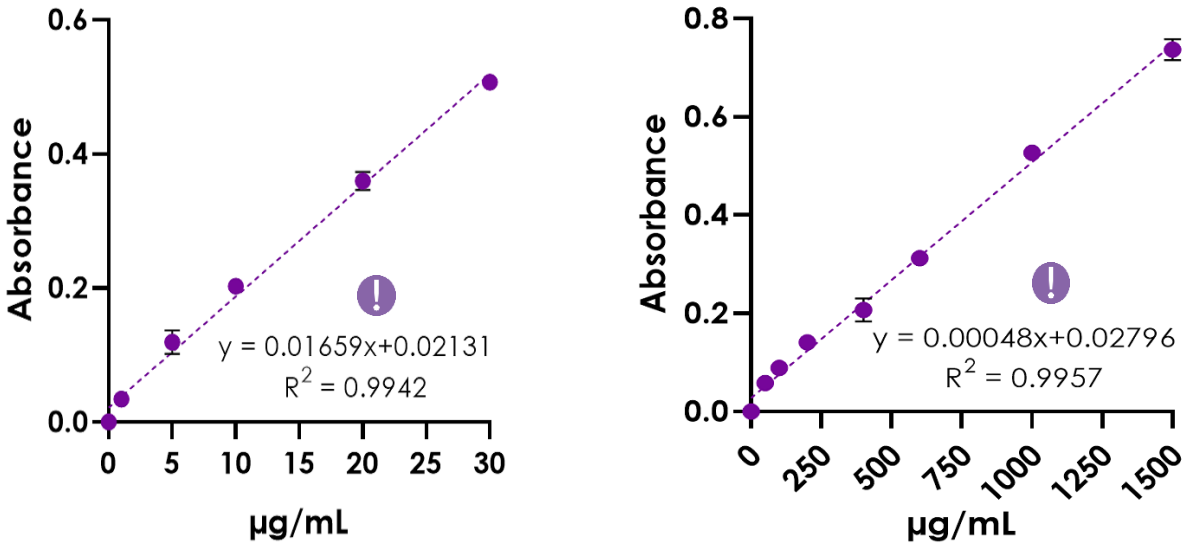
3  **Incubate** the mixture for **at least 5 minutes** at **RT** The incubation step should not be longer than one hour

4  Read the **absorbance** at **595 nm**

9. Data analysis

ANALYSIS OF THE STANDARDS

- Calculate the average absorbance of the standards.
- Subtract the average absorbance of the reagent blank (Std 1) from the average absorbance of the standards to obtain the blank-corrected absorbance of the standards.
- Create a standard curve by plotting the blank-corrected absorbance of the standards as a function of the standard concentration (see **STANDARD CALIBRATION** section). Typical standard curves (**y**=slope·**x** ± intercept) for the microplate assay procedures are shown below.



Protein standard curve with Bradford Protein Quantification Assay Kit: low protein concentration (left) and high protein concentration (right)

This standard curve is an example of the data typically obtained with this kit. **DO NOT USE** this standard curve to calculate the protein concentration of your samples. A new standard curve must be performed by the end user.

ANALYSIS OF THE SAMPLES

- Calculate the average absorbance of the samples.
- Subtract the average absorbance of the reagent blank (Std 1) from the average absorbance of each sample to obtain the blank-corrected absorbance of the samples (A_S).
- Calculate the protein concentration of the samples using the following equation. Slope and intercept values are obtained from the standard curve.

$$\text{Protein (µg/mL)} = \left(\frac{A_S - \text{intercept}}{\text{slope}}\right)$$

When working with diluted samples the concentration values obtained must be multiplied by the dilution factor to obtain the protein concentration of the undiluted sample.

10. Troubleshooting

This troubleshooting table provides potential sources and solutions for common problems observed with BQC Assay Kits. **The problems listed below could occur when using any BQC Assay Kit**. They are not specific for this assay kit.

Problem	Possible Cause	Recommended Solution
Wells have color but there is no reading	Plate read at incorrect wavelength	Check the wavelength used in the assay
	Incorrect microplate	Use the correct microplate for your application UV/Vis: transparent Fluorescence: black wells/transparent bottom
Standard readings do not follow a linear pattern	Pipetting errors in preparation of standards	Avoid pipetting small volumes (<5 µL) Be careful not to splash from well to well
	Air bubbles formed in well(s)	Use reverse pipetting technique
	Standard stock is at incorrect concentration	Always refer to dilutions described in **Assay preparation**
	Improperly thawed reagents	Thaw all components completely and mix well before use
	Use of improperly stored reagents	Store the components appropriately Use fresh components from the standard curve
	Incorrect incubation times or temperatures	Refer to **Assay protocol**
Dispersion of standard and sample readings	Pipetting errors	Avoid pipetting small volumes (<5 µL) Be careful not to splash from well to well
	Air bubbles formed in well(s)	Use reverse pipetting technique

Problem	Possible Cause	Recommended Solution
Sample erratic values	Samples contain interfering substances	Dilute sample further (if possible)
	Inappropriately stored samples or samples used after multiple freeze-thaw cycles	Use fresh samples or store appropriately until use
	Samples not deproteinized	Use an appropriate deproteinization protocol
	Cells/Tissue samples not homogenized completely	Repeat the sample homogenization
	Inappropriate sample dilution buffer	Refer to **Assay preparation**
Sample reading fall outside the detection range	Samples are too diluted/concentrated No analyte/activity is observed in the sample	Re-assay using different sample dilutions

STILL HAVING PROBLEMS?

Contact BQC if you have any further questions, our team will be pleased to help you:

	Phone	+ 34 985 26 92 92
	E-mail	info@bioquochem.com
	Business hours	Monday-Thursday: 8.30 to 17.00 (CEST) Friday: 8.00 to 15.00 (CEST)

11. Additional information

Bradford Protein Quantification Assay Kit is a quick (< 15 minutes), simple and precise (RSD < 5 %) assay for determining proteins in a wide variety of samples.

Surfactants at concentrations routinely used to solubilize membrane proteins have been reported to interfere with this assay. Proteins with poor acid-solubility cannot be measured with this assay.

If unexpected results are obtained running your samples, please contact us at info@bioquochem.com

12. Related products

More products available on bioquochem.com

Reference	Product
KB03026	TCA Deproteinizing Assay Kit
KF01005	ORAC Antioxidant Capacity Assay Kit
KB03008	Protein Carbonylation Assay Kit

13. Warranties and limitation of liability

BQC shall not in any event be liable for incidental, consequential or special damages of any kind resulting from any use or failure of the products, even if BQC has been advised of the possibility of such damage including, without limitation, liability for loss of use, loss of work in progress, downtime, loss of revenue or profits, failure to realize savings, loss of products of buyer or other use or any liability of buyer to a third party on account of such loss, or for any labor or any other expense, damage or loss occasioned by such product including personal injury or property damage is caused by BQC´s gross negligence. Any and all liability of BQC hereunder shall be limited to the amounts paid by the buyer for the product.

Buyer´s exclusive remedy and BQC´s sole liability hereunder shall be limited to a refund of the purchase price, or the replacement of all material that does not meet our specifications.

Said refund or replacement is conditioned on buyer giving written notice to BQC within 30 days of shipment.

Expiration date: 1 year from the date of fabrication. Expiration date is indicated on the outside of the box.

For further details, please refer to our website **bioquochem.com**

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REDOX TECHNOLOGIES

Edificio CEEI | Parque Tecnológico de Asturias,

33428 Llanera, Asturias

Info@bioquochem.com

www.bioquochem.com