MTT CELL PROLIFERATION ASSAY

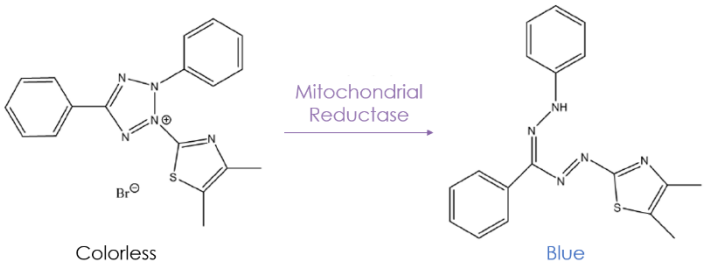
KF03001-A/B-500/2000/5000 Tests

DESCRIPTION AND USE

Cell proliferation has been shown to have multiple functions in development and pattern formation, including roles in growth, morphogenesis, and gene expression. Methods commonly used for this purpose are hemocytometer counting, determination of protein content, wet or dry weight measurement, and determination of the optical density (OD). While hemocytometer counting, and protein determination have the disadvantage of being time-consuming and tedious, the measurement of wet or even dry weight is not practical for very small culture volumes.

An alternative method is based on the transformation and colorimetric quantification of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide. The respiratory chain and other electron transport systems reduce MTT and other tetrazolium salts and thereby form non-water-soluble violet formazan crystals within the cell. The amount of these crystals can be determined spectrophotometrically and serves as an estimate for the number of mitochondria and hence the number of living cells in the sample. These features can be taken advantage of in cytotoxicity or cell proliferation assays, which are widely used in immunology, toxicology, and cellular biology.

The principle of the MTT Cell Proliferation Assay is based in mitochondrial activity. For viable cells, mitochondrial activity is constant and thereby an increase or decrease in the number of viable cells is linearly related to mitochondrial activity. The mitochondrial activity of the cells is reflected by the conversion of the tetrazolium salt MTT into formazan crystals, which can be solubilized for homogenous measurement. Thus, any increase or decrease in viable cell number can be detected by measuring formazan concentration spectrophotometrically using a plate reader at 570 nm vs. 690 nm.



Scheme 1. Reduction of MTT to formazan

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In the **MTT-A** Cell Proliferation Assay is necessary to remove the cell medium before resuspending MTT crystals formed.

In the **MTT-B** Cell Proliferation Assay the resulting formazan crystals are directly redissolved without removing the culture medium. This kit is suitable for cells in suspension and faster, but less sensitive, than MTT Cell Proliferation Assay A.

MATERIALS SUPPLIED

BQC MTT Cell Proliferation Assay contains

MTT-A (KF03001-A)

Item	No. Tests	Quantity	Storage
MTT Solution	500	5 vials	-20 °C
	2000	1 bottle	
	5000	1 bottle	
MTT Solvent	500	1 bottle	RT
	2000	2 bottles	
	5000	1 bottle	

MTT-B (KF03001-B)

Item	No. Tests	Quantity	Storage
MTT Solution	500	5 vials	-20 °C
	2000	1 bottle	
	5000	1 bottle	
MTT Solvent	500	1 bottle	RT
	2000	2 bottles	
	5000	1 bottle	

STORAGE AND STABILITY

On receipt store kit components as indicated above. Do not use after the expiration date stated on the packaging.

SAMPLE PREPARATION

This protocol is for a 96-well format. Volumes of culture cells, media and reagents may differ from the format described below.

1. Add 100 µL of culture cells to each well at an appropriate density. Include one set of wells with medium but no cells (control).
2. Incubate the cells overnight.
3. Treat cells on day two with agonist, inhibitor or drug (V_f= 100 µL) or change culture media if no treatments are required.
4. After the incubation time (drug and cell-dependent), follow the protocol described in the Assay Protocol section.

ASSAY PROTOCOL

After sample preparation, follow next steps:

NOTE: These volumes are for a 96-well plate. For other sizes extrapolate the reagents volume.

1. Add 10 µL of MTT Solution to each well (10% of the culture media volume).
2. Incubate for 4 hours at 37 °C in a culture hood. The optimal incubation time may differ in each assay.

From here on, there are two different protocols depending on the reference:

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KF03001 A:

3. After the incubation period, remove culture medium from the culture hood and dissolve the resulting formazan crystals.
    a. If cells are attached to culture vessels growth surface, remove and discard the culture media. Add MTT Solvent in an amount equal to the original culture volume.
    b. If cells are not attached, add MTT Solution directly to the culture media in an amount equal to the original culture volume.
4. Cover and agitate 96-well plate on an orbital shaker for 15 minutes. (Within 1 hour of MTT Solvent addition).
5. Read absorbance at 570 nm.

KF-03-001 B:

3. After the incubation period, dissolve the resulting formazan crystals with the MTT Solvent in an amount equal to the culture volume.
4. Cover and agitate 96-well plate on an orbital shaker for 15 minutes. (Within 1 hour of MTT Solvent addition).
5. Read absorbance at 570 nm.

RELATED PRODUCTS

Product	Reference
Resazurin Based Cell Viability Assay	KC04002
MTT-A Cell Proliferation Assay	KF03001A
DNA Quantification Kit – Hoechst Assay	KC04003

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