Cell Growth Determination Kit (MTT)

KB-03-001

500 tests (96 well plate)

Index

Introduction

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[1].The respiratory chain[2] and other electron transport systems[3] 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[4]. These features can be taken advantage of in cytotoxicity or cell proliferation assays, which are widely used in immunology, toxicology, and cellular biology[5].

Materials

Bioquochem MTT **Kit** contains:

Product	Quantity	Storage
MTT Solution	5 vials	-20ºC
MTT Solvent	1 bottle	Room Temperature

- **This kit is for R&D use only**

All these chemicals should be handled with care

Assay principle

The principle of the MTT 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.

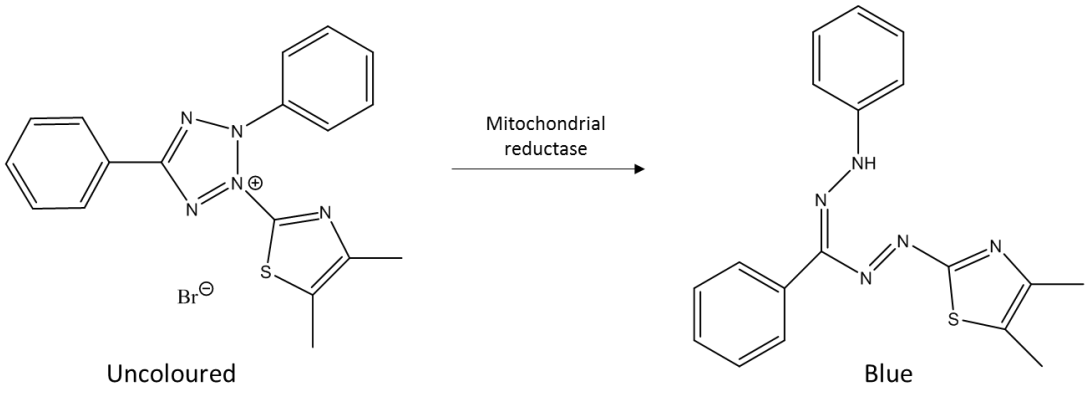

Figure 1. Reduction of MTT to formazan

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 appropiate 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 Assay Protocol section.

Assay Protocol

Performing the assay

After sample preparation, follow next steps:

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 (see cell images in our web page).
3. After the incubation period, remove cells from the culture hood and dissolve the resulting formazan crystals [a,b].
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.

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.

References

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3. Liu, Y., Peterson, D. A., Kimura, H. & Schubert, D. Mechanism of cellular 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction. *J. Neurochem.***69,** 581–93 (1997).
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