CellQuanti-MTT™ Cell Viability Assay Kit (CQMT-500)

Non-radioactive Colorimetric Assay for Cell Proliferation and Cytotoxicity

DESCRIPTION

The study of cell proliferation and cell viability requires the accurate quantification of the number of viable cells in a cell culture. Therefore, assays for calculating cell viability are necessary for optimizing cell culture conditions, evaluating cell growth factors and nutrients, discovering novel antibiotics and anti-cancer drugs, evaluating toxic effects of environmental pollutants and cell mediated toxicity and studying programmed cell death (apoptosis).

The CellQuanti-MTT™ assay kit provides a convenient, sensitive, quantitative and reliable assay for determining the number of viable cells in a given culture. This homogeneous colorimetric assay is based on the conversion of a tetrazolium salt MTT, a pale yellow substrate, to formazan, a purple dye. This cellular reduction reaction involves the pyridine nucleotide cofactors NADH/NADPH and is only catalyzed by living cells. The formazan product has a low aqueous solubility and is present as purple crystals. Dissolving the resulting formazan with a solubilization buffer permits the convenient quantification of product formation. The intensity of the product color, measured at 550 - 620 nm, is directly proportional to the number of living cells in the culture. Reagents in the kit have been carefully formulated and optimized for sensitivity, assay robustness and automation.

KEY FEATURES

Safe. Non-radioactive assay.

Sensitive and accurate. As low as 950 cells can be accurately quantified..

Convenient and high-throughput. "Mix-incubate-measure" type assay. No wash and reagent transfer steps are involved. Z' factors of 0.5 and above are observed. Can be readily automated with HTS liquid handling systems.

APPLICATIONS

Cell Proliferation: effects of cytokines, growth factor, nutrients.

Cytotoxicity and Apoptosis: evaluation of toxic compounds, anti-cancer antibodies, toxins, environmental pollutants etc.

Drug Discovery: high-throughput screen for toxic and anticancer drugs..

KIT CONTENTS

Reagent: 10 mL **Solubilizer:** 50 mL

Control Reagent (Cat # **CTTX-050**): 50 mg saponin (sold separately).

Storage conditions: The kit is shipped at room temperature. Store the Reagent at -20 °C. Solubilization Solution can be stored at room temperature. Shelf life: 12 month after receipt.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

ASSAY PROCEDURES

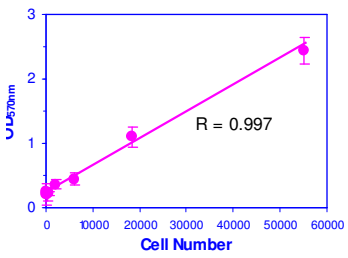
1. Plate and culture cells (80 µL per well) in a clear bottom 96-well tissue culture plates. Assays can be performed on either adherent cells or cells in suspension. The number of cells can vary from 1,000 to 80,000 per well. The volume can vary from 50 to 150 µL, although 80 µL is used in this example. In addition to the test samples, one must include control wells of culture medium containing no cells or cells treated with a toxic reagent such as 0.1% saponin.
2. Add test compounds and controls and incubate cells for the desired period of time (typically overnight). It is recommended that assays be run in duplicate or triplicate. A volume of 20 µL in phosphate buffered saline (PBS) or culture medium is recommended for the test compounds and controls. The Control Reagent can be conveniently reconstituted with 5 mL PBS (1% saponin).
3. Warm Reagent and to room temperature. Add 15 µL (per 80 µL cell culture) of Reagent per well and incubate for 4 hours at 37°C. The volume of the reagent should be adjusted depending on the volume of cell culture.
4. Add 100 µL of the Solubilizer to each well. Mix gently on an orbital shaker for one hour at room temperature. The volume of the Solubilizer should be adjusted depending on the volume of cell culture. If precipitation occurs in the Solubilizer, place the bottle in a warm water bath or at 37°C and shake to dissolve precipitates.
5. Measure OD_{570nm} for each well on an absorbance plate reader. Maximum absorbance of the formazan dye lies between 560 and 590 nm. If desired, the OD measurement can be performed the following day. In this case, it is recommended to seal the plate to minimize evaporation.

DATA ANALYSIS

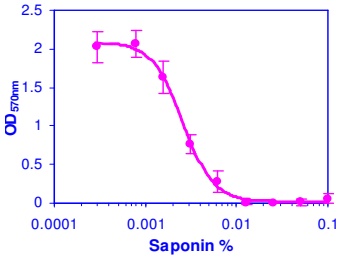
Determine the average of the blank controls and subtract this amount from all absorbance values. Plot the corrected absorbance values at 570 nm against the concentration of the test compound. Determine the EC_{50} value for cell proliferation and IC_{50} value for cytotoxic compound by non-linear regression analysis using Prism or another data analysis tool.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices, clear 96-well culture plates (e.g. VWR cat# 82050-760), and plate reader capable of either measuring absorbance between 560-590 nm.



HEK293 Cell Titration



Saponin Titration (5.5 × 10^4 HEK293 Cells per well) The IC_{50} for saponin was 0.0026 wt%.

PUBLICATIONS

1. Zhang, Y et al (2009). Estrogen inhibits glucocorticoid action via protein phosphatase 5 (PP5)-mediated glucocorticoid receptor dephosphorylation. J Biol Chem.284 (36):24542-52. Assay: Cell Viability in Human MCF-7 cells.
2. Oxelmark, E et al (2006). The cochaperone p23 differentially regulates estrogen receptor target genes and promotes tumor cell adhesion and invasion. Mol Cell Biol. 26(14):5205-13. Assay: Cell Viability in Human cell lines.
3. Gupta, V et al (2009). Fabrication and characterization of silk fibroin-derived curcumin nanoparticles for cancer therapy. Int. J. Nanomedicine 2009:4 115–122. Assay: Cell Viability in Human cells.
4. Cheng, H et al (2010). Transient receptor potential melastatin type 7 channel is critical for the survival of bone marrow derived mesenchymal stem cells. Stem Cells Dev. 19(9):1393-403. Assay: Cell Viability in Mouse mesenchymal stem cells.
5. Chen, TS et al (2010). Delineating biological pathways unique to embryonic stem cell-derived insulin-producing cell lines from their noninsulin-producing progenitor cell lines. Endocrinology 151(8):3600-10. Assay: Cell Viability in Mouse stem cells.
6. Desplats, PA et al (2008). Functional roles for the striatal-enriched transcription factor, Bcl11b, in the control of striatal gene expression and transcriptional dysregulation in Huntington's disease. Neurobiol Dis. 31(3):298-308. Assay: Cell Viability in Human cells.