SciénCell™
Research Laboratories

MTT Cell Viability & Proliferation Assay

Cat. No. 8028
1000 Tests in 96-well plate

Introduction

The study of cell viability and proliferation is very important for evaluating a cell population's responses to external factors, such as growth factors, antibiotics and anti-cancer drugs. The ScienCell™ MTT Cell Viability & Proliferation Assay allows simple, accurate and reliable counting of metabolically active cells, based on the conversion of pale yellow tetrazolim MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] to purple formazan crystals. The crystals can be solubilized and then spectrophotometrically quantified.

Kit Components

Cat. No.	# of vials	Reagent	Quantity	Storage
8028a	5	MTT powder	10 mg	4°C
8028b	2	MTT Solubilization Buffer	50 ml	4°C

Quality Control

Human Vessel Umbilical Endothelia Cells (Cat. No. 8000, ScienCell™) serial diluted are plated into 96-well plate. The MTT Cell Viability & Proliferation Assay is applied and a linear relationship can be observed between signal produced (OD_{570nm}- OD_{690nm}) and the number of cells (Figure 1).

Procedures (96-well plate)

1. Plate and culture cells in a clear-bottom 96-well tissue culture plate. Incubate cells with test compounds and controls for the desired period of time. The final volume of culture medium in each well should be 100 µl.
2. Reconstitute each vial of MTT with 2 ml of PBS, pH 7.4. Vortex briefly, sterile filter and keep in the dark at 4°C until use. Fresh reconstitution of MTT is recommended for each experiment, although reconstituted MTT solution should be stable for up to 2 weeks when kept at 4°C, protected from light.
3. Equilibrate the MTT Solution to room temperature, and then add 10 µl of MTT Solution to each well (the volume of MTT solution should be 1/10 of the original culture medium). Mix well by gently rocking the plate side-to-side.
4. Incubate cultures with MTT at 37°C for 2-4 hours depending on cell type and seeding density. At the end of incubation, there should be black crystals formed in the live cells.
5. After incubation, add 100 µl of MTT Solubilization Buffer (equal to the volume of original culture medium) to each well and pipette up and down to help dissolve crystals. Gentle mixing on an orbital shaker will further enhance dissolution.
6. Within an hour, measure the absorbance on an ELISA plate reader with a test wavelength at 570 nm and a reference wavelength at 690 nm, and subtract the 690 nm background absorbance from the 570 nm measurement.

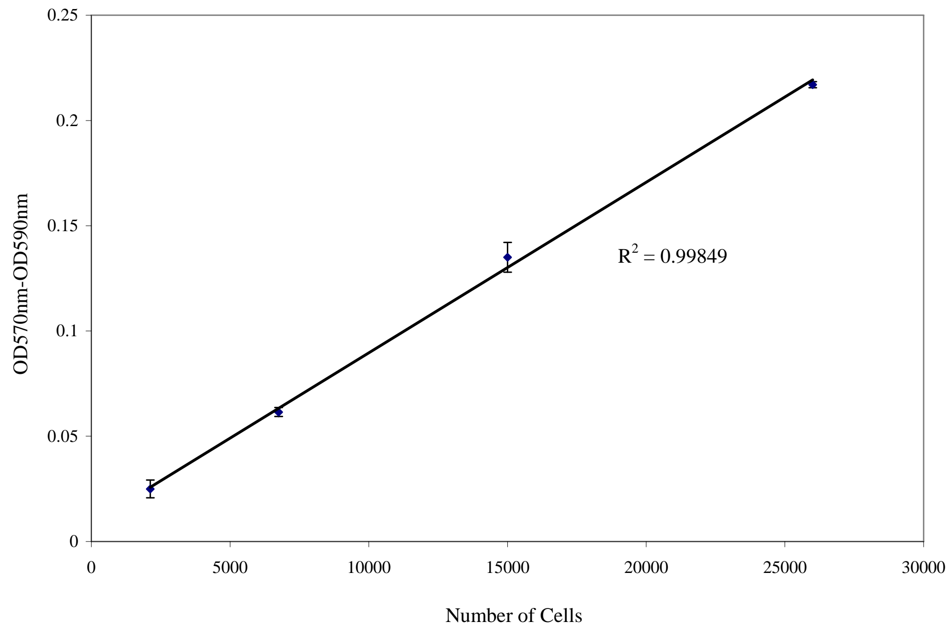

Figure 1. A linear relationship can be observed between OD_{570nm}-OD_{690nm} and the number of HUVECs.