BIOMEDICAL RESEARCH SERVICE CENTER
UNIVERSITY at BUFFALO, STATE UNIVERSITY of NEW YORK

Department of Biochemistry, Attn: Dr. Lee, University at Buffalo, 3435 Main Street, Buffalo, NY 14214, USA
Tel/Fax: (716) 829-3106 Email: chunglee@buffalo.edu Web: www.bmrservice.com

Cell Viability/Proliferation Assay Kit (Cat #: A-100)

COMPONENTS: MTT Solution- 50 ml, store at -20℃ (for 3000 – 5000 wells)

PRODUCT DESCRIPTION: The use of a colorimetric assay for cell growth, proliferation, and survival offers major advantages in speed, cost, and safety over other assays relying on the use of radioactive compounds (such as ^{3}H-thymidine or ^{51}Cr). One of the popular methods developed toward this end is the MTT assay, which relies on the ability of live but not dead cells to reduce a water-soluble yellow dye, MTT or 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide, to a water-insoluble purple formazan product (J. Immunol. Methods 65:55, 1983 & Cancer Res. 47:936,1987). Active mitochondrial dehydrogenases of living cells are believed to cause this conversion. The water insoluble formazan can be solubilized using DMSO or isopropanol, and the dissolved material is measured spectrophotometrically yielding absorbance as a function of concentration of converted dye. The assay requires minimal manipulation, is easily automated, and thus is ideally suited to a program that involves a wide range of cell types and thousands of potential drug agents. Both monolayer and suspension cell cultures can be assayed using the MTT conversion method. The MTT solution is stable for many years if handled and stored properly.

PROTOCOL

Adherent cells

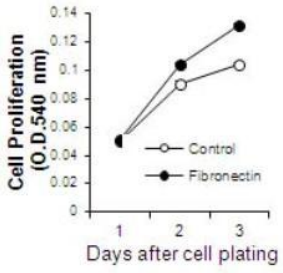
1. Preparation of working MTT solution: Prepare fresh a working MTT solution by mixing 1 part of MTT Solution with 20 − 40 parts of a cell growth medium in a culture tube (use 40 parts if dye conversion is too intense). The dilution factor can be adjusted empirically to obtain a more linear response for each cell system.

2. MTT dye conversion: Aspirate off culture medium from the wells or dishes. Add 0.1 ml of the freshly prepared working MTT solution to each well of a 96-well plate or 0.3 ml to each well of a 24-well plate. Adjust volume accordingly for a different surface area. Incubate cells in a 37°C CO_2 incubator for 20 min. Note that since mitochondrial abundance affects the MTT dye conversion rate, the incubation time may be adjusted empirically to obtain a more linear response. The soluble yellow MTT dye is converted to an insoluble purple formazan during incubation.

3. Dye extraction and quantification: Aspirate off working MTT solution completely. Add 0.1 − 0.3 ml of DMSO to each well, making sure the cells are fully immersed in DMSO. Agitate DMSO solution for ~2 min to ensure complete dye extraction. Transfer DMSO extract to a new 96-well plate. Measure absorbance at 540 – 570 nm using DMSO as blank. The absorbance is a function of cell viability and cell proliferation rate, assuming the mitochondrial content is not affected by the experimental condition. Plot the data as illustrated, which shows that a fibronectin substratum increases mesenchymal cell proliferation.

Suspension cells

Add MTT Solution (not working MTT solution) directly to the cells being assayed. MTT solution should have been diluted by the cell growth medium 20 – 40 fold after addition. Immediately mix the cell suspension to ensure complete dye dispersion. After 20-min incubation in a 37°C CO_2 incubator for dye conversion, spin down cells and aspirate off medium. Add enough DMSO to solubilize the purple formazan by vigorous vortexing. Spin down insoluble debris briefly and measure absorbance at 540 – 570 nm using DMSO as blank in a 96-wll plate. Note that the MTT dilution factor, dye conversion time and volume of DMSO should be adjusted empirically to obtain a more linear response.



Additional Information:

- Dimethyl Sulfoxide (DMSO) is not provided in the kit, and is required for dye extraction.
- MTT solution is prepared in 70% ethanol. Please contact us or visit the product webpage for MSDS information on ethanol, DMSO, and MTT.