BIOMEDICAL RESEARCH SERVICE, UNIVERSITY AT BUFFALO

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

COMPONENTS: MTT Solution- 50 ml, store at -20ºC

PRODUCT DESCRIPTION: The Cell Proliferation/Viability Assay Kit is based on the ability of live cells to reduce a water-soluble tetrazolium dye (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) to a water-insoluble formazan product (J. Immunol. Methods 65:55, 1983). Active cellular dehydrogenases of living cells are believed to cause this conversion. The insoluble formazan can be solubilized using DMSO for quantitative measurement. Both monolayer and suspension cell cultures can be assayed. The MTT solution is stable for many years if handled and stored properly.

PROTOCOL

Adherent (monolayer) cells:

1. Prepare a fresh working solution by mixing 1 part of MTT Solution with 40 parts of a cell culture medium. Use the working solution immediately. Discard unused solution.

2. Aspirate off culture medium from culture vessel. Add 0.1 ml working solution to each well of a 96-well plate or 0.2 ml to each well of a 24-well plate.

3. Incubate cells in a 37°C CO_2 incubator for 20 min. The MTT dye is converted to the water insoluble purple formazan during incubation.

4. Aspirate off working solution completely. Add 0.1 ml DMSO to each well of a 96-well plate or 0.2 ml to each well of a 24-well plate, making sure the cells are fully immersed in DMSO. Gently agitate plate for 2 min to ensure complete dye solubilization.

5. Transfer dye solution to a plain (uncoated) 96-well plate. Add an equal volume of DMSO to an empty well as blank. Measure optical density (O.D.) at 540 – 570 nm using a plate reader. Subtract the DMSO well reading from each sample well reading to generate ΔO.D., which is a function of cell proliferation rate or cell viability.

Non-adherent (suspension) cells:

1. Add 5 µl of MTT Solution to each 1.5-ml microtube.

2. Add 200 µl of each cell suspension to each microtube containing 5 µl of MTT Solution. Immediately vortex tube for 2 sec after mixing.

3. Incubate tubes uncapped in a 37°C CO_2 incubator for 20 min.

4. After 20-min incubation, centrifuge tubes in a microfuge at maximal speed (~13,000 rpm) for 3 min. Aspirate off supernatant from each tube.

5. Add 0.2 ml DMSO to each tube. Solubilize the purple dye by vigorous vortexing for at least 5 min.

6. Centrifuge tubes in a microfuge at maximal speed (~13,000 rpm) for 3 min. Transfer supernatant to a plain (uncoated) 96-well plate. Add 0.2 ml DMSO to an empty well serving as blank.

7. Measure optical density (O.D.) at 540 – 570 nm using a plate reader. Subtract the DMSO well reading from each sample well reading to generate ΔO.D., which is a function of cell proliferation rate or cell viability.

Additional Information:

- Dimethyl sulfoxide (DMSO) is not provided with the kit and is required for dye extraction. Please contact us or visit the product webpage for SDS information on ethanol, DMSO, and MTT.