MTT Cell Proliferation and Cytotoxicity Assay Kit

Catalogue No.:abx090676

MTT Cell Proliferation and Cytotoxicity Assay Kit is an assay kit for detecting cell proliferation and cytotoxicity. MTT [3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide], a yellow tetrazole, is reduced to an insoluble purple crystal formazan by mitochondrial dehydrogenases. The O.D. value can be read at 570 nm; high O.D.s indicate cell proliferation whilst low O.D.s indicate cell cytotoxicity. When using the provided Formazan Diluent Buffer, it is not necessary to remove the intrinsic cell culture medium, and therefore unnecessary assay error can be avoided.

Kit Components:

- MTT Staining Solution: 5 ml
- Formazan Diluent Buffer: 55 ml

Target:	MTT Cell Proliferation and Cytotoxicity
Form:	Liquid
Storage:	Store the MTT Staining Solution at -20 °C in the dark for up to one year. Store the Formazan Diluent Buffer between 2-8°C for up to one year.

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Directions for use:

Protocol

1. Bring all reagents to room temperature before use.
2. Collect logarithmic phase cells and adjust the cell suspension concentration. Add 100 µl into each well of a cell-culture plate and adjust the cell density to 1000-10,000 cells per well (sterile PBS should be added to the edge of the wells of the plate).
3. Seed cells in a 5% CO_2 incubator in the dark at 37 °C until cells are uniform at the bottom of the wells. The cell number for each well should be determined by cells' size and proliferation rate. Add 0-10 µl of the compound of interest per well (for a concentration gradient, this can be added after cells adhere, or every 2 hours, or every half-day, etc). It is recommended to set 5 wells to analyse in duplicate.
4. Incubate the cells for 16-48 hours, then observe under microscope by inverting the cells.
5. Add 10 µl of MTT Staining Solution into each well. Culture for 4 hours. If the compound of interest can react with MTT, centrifuge and remove the cultured medium. Rinse with PBS carefully 2-3 times, then add the MTT-containing cultured medium.
6. Add 100 µl of Formazan Diluent Buffer into each well, then mix gently at low speed for 10-30 min until the crystal is dissolved completely. If undissolved Formazan is still present, it is recommended to pipette up and down gently, avoiding bubbles, 2-3 times, or incubate overnight. Measure the O.D. value at 570 nm.
7. Set blank wells (cell culture medium, MTT Staining Solution, Formazan diluent buffer) and control wells (cells, media containing the compound of interest with the same concentration, culture medium, MTT Staining Solution, Formazan diluent buffer).

Notes:

• Ensure that the wells do not dry out due to evaporation, particularly when the cell culture time is long. Avoid using the outermost wells; use PBS, water or culture medium to replace any liquid that has evaporated; or place the 96-well plate near water in the incubator.
• The MTT Staining Solution should not be used if it has turned a gray-green colour.
• If a precipitate forms in the Formazan Diluent Buffer, heat using a 37 °C water bath until the precipitate dissolved.
• Incubation steps should be carried out in the dark.
• Wear Personal Protective Equipment (PPE), such as lab coats, lab glasses and gloves, when using this product.

Note: This product is for research use only.

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