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MTT Cell Proliferation and CytotoxicityAssay Kit

Cat Number: AK0948 Package: 500T/1000T

Storage: MTT Solution stored at -20°C; Formazan Dissolving Solution stored at room temperature

or -20 $^{\circ}$ C condition.

Kit Components

Component	500T	1000T
MTT Solution	5mL	5mL×2
Formazan Dissolving Solution	60mL	60mL×2

Product Description

The kit is designed for the spectrophotometric measurement of cell growth as a function of mitochondrial activity in living cells. Using MTT could measure the activity of living cells via mitochondrial dehydrogenase activity with simple, accurate and reproducible functions. Dehydrogenases in mitochondria can deoxidize MTT to crystalline purple product Formazan. The crystals can be dissolved in specific solvent. Use Microplate Absorbance Reader to measure absorbance near 490nm wavelength. An increase in cell number results in an increase in the amount of MTT Formazan formed and an increase in absorbance.

For best results, cell numbers should be determined during log growth stage. Each test should include a blank containing complete culture medium without cells.

Procedure

- 1. Harvest cell(log growth stage) adjust the concentration of cell suspension, separate into 96 well plate, 180μL/well, 3000-10000 cell/well.
- 2. Return cultures to incubator $(37^{\circ}\text{C},5\%\text{CO2})$ and incubate for 6 to 24 hours.
- 3. Add appropriate concentrations of toxicant into the culture media in the plate. Incubate the plate for an appropriate length of time in the incubator.
- 4. Remove supernatant, add 90ul fresh culture medium then add 10ul MTT Solution then continue to culture for 4h.
- 5. Remove supernatant, each well added with 110µL Formazan Dissolving Solution, Slowly stirring in a gyratory shaker for 10 min, make the crystal is fully dissolved. Measure the absorbance at 490 nm using a microplate reader.
- 6. Meanwhile set the zero adjustment holes (Culture medium, MTT Solution, Formazan Dissolving Solution), control holes (Cell, solvent of the same concentration toxican, Culture medium, MTT Solution, Formazan Dissolving Solution), each group set 3 holes.

Notes:

- 1. Uneven evaporation of culture fluid in 96well plates may cause erroneous results. The outermost circle is most easy to evaporate, so you can discard them.
- 2. MTT Solution is stable when stored frozen. Storage at 2-8 °C may result in decomposition



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- and yield erroneous results. MTT Solution should be storage avoid light, as MTT will invalidation when light for a long time.
- 3. Development of dark color or formation of crystals indicate product deterioration. If the Formazan Dissolving Solution is frozen or precipitate can be dissolved in 37 °C water bath, the precipitate must be dissolved and mixed before use.