

MTT Cell Proliferation Assay Kit

Cat. No. 21180 1000 rxn.

■ Description

MTT Cell Proliferation Assay Kit provides a simple method for determination of cell number using standard microplate absorbance readers. Determination of cell growth rates is widely used in the testing of drug action, cytotoxic agents and screening other biologically active compounds. Several methods can be used for such determinations, but indirect approaches using fluorescent or chromogenic indicators provide the most rapid and large scale assays. Among such procedures, the MTT assay is still among one of the most versatile and popular assays.

■ Contents and Storage Condition

- MTT solution
- Solubilization solution 1ml x 10 vials (10 ml)
- Storage condition 50 ml x 2 bottle (100 ml)
 - : Stable at -15 to -25°C until the expiration date printed on the label. After thawing, the MTT labeled reagents must be stored at 4°C in the dark.
 - : Protect from light. Repeated thaw-freeze cycles do not affect product stability

■ Characteristic

- No radioactive isotopes.
- The absorbance revealed, strongly correlates to the cell number.
- Low cell numbers detection.
- Use of multiwell-ELISA readers.
- No washing steps and no additional reagents.

■ Materials Required But Not Provided

- Phosphate-buffered saline (PBS), sterile
- Dimethylsulfoxide (DMSO) - optional

■ Protocols

1. Cells are grown in microplates (tissue culture grade, 96 wells, flat bottom) in a final volume of 200 μ l culture medium per well, in a humidified atmosphere (e.g. 37°C, 5% CO₂). The incubation period of the cell cultures depends on the particular experimental approach and on the cell line used for the assay.
2. After the incubation step, add 10 μ l of the MTT solution to each well.
3. Incubate the microplate for 4 hrs in a CO₂ incubator (e.g. 37°C, 5% CO₂).
Note: More than 4 hrs incubation will decrease the sensitivity of the assay.
4. Carefully remove media. Do not disturb cells and do not rinse with PBS. **Note:** For non-adherent cells it may be necessary to first centrifuge the plates to sediment the cells.
5. Add 100 μ l of the Solubilization solution into each well.
Note: To shorten the time of the assay it is possible to use DMSO (not provided) as a solubilizing agent to dissolve the formazan.
6. Check for complete solubilization of the purple formazan crystals and measure the absorbance of the samples using a microplate (ELISA) reader. The wavelength to measure absorbance of the formazan product is between 550 and 600 nm according to the filters available for the ELISA reader, used.

■ Notice

1. Aseptically add MTT solution in an equal culture volume.
2. Incubation times should be consistent when making comparisons.
3. If cells are attached to culture vessel growth surface, remove and dispose of the culture fluid. Add MTT solution in an equal amount to the original culture volume. Solvent volumes may vary but the final volumes should be consistent to facilitate comparison.
4. If cells are not attached or loss of MTT formazan occurs if culture fluid is removed, add MTT solvent directly to the culture in an amount equal to the original culture volume. Plates should be read within 1 hour after adding MTT solvent.



5. Gentle shaking will enhance dissolution. Occasionally, pipetting up and down (trituration) may be required to completely dissolve the MTT formazan crystals especially in dense cultures.

5. Microbial contamination will contribute to the cleavage of MTT and formation of MTT formazan yielding erroneous results.

6. Uneven evaporation of culture fluid in wells of multiwell plates may cause erroneous results.

■ MTT assay principle

Trypan blue staining is a simple way to evaluate cell membrane integrity (and thus assume cell proliferation or death) but the method is not sensitive and cannot be adapted for high throughput screening. Measuring the uptake of radioactive substances, usually tritium-labeled thymidine, is accurate but it is also time-consuming and involves handling of radioactive substances. The MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a tetrazole) is reduced to purple formazan in the mitochondria of living cells. The absorbance of this colored solution can be quantified by measuring at a certain wavelength (usually between 550 and 600nm) by a spectrophotometer. The absorption max is dependent on the solvent employed. This reduction takes place only when mitochondrial reductase enzymes are active, and therefore conversion can be directly related to the number of viable (living) cells. When the amount of purple formazan produced by cells treated with an agent is compared with the amount of formazan produced by untreated control cells, the effectiveness of the agent in causing death of cells can be deduced, through the production of a dose-response curve.

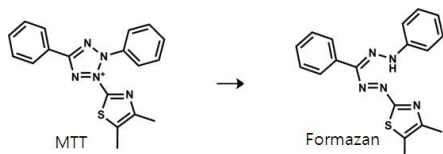


Fig 1. Metabolization of MTT to a formazan salt by viable cells

TECHNICAL DATA

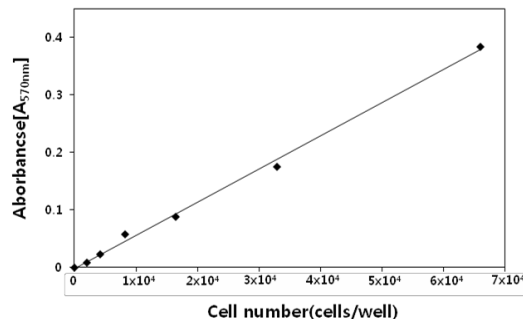


Fig 2. Effect of different numbers of cells on color formation.

RELATED PRODUCTS

Product Name	Cat. No.
iN-fect™ in vitro Transfection Reagent	15081
iN-fect Miracle™ Transfection Reagent	11062
iN-fect BOND™ Cell Adhesion Reagent	15082
PBS 1X, 10X, 20X	IBS-CB013 / IBS-CB014 / IBS-CB015

