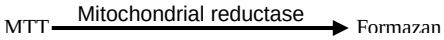
MTT Cell Proliferation Assay Kit (Colorimetric)

(Catalog # NBP2-54883 Store kit at -20°C)

I. Introduction:

Quantification of the number of viable cells is an indispensable tool in cell biology research. Novus' MTT Cell Proliferation Assay Kit is a sensitive method for quantification of viable cells in proliferation and cytotoxicity assay. The method is based on the conversion of water soluble MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) compound to an insoluble formazan product. Viable cells with active metabolism convert MTT into formazan, however, dead cells lose this ability. Thus color formation serves as a useful and convenient marker of only the viable cells. The measured absorbance (590 nm) is proportional to the number of viable cells. This assay kit provides an easy-to-use, non-radioactive, and high-throughput method for cell proliferation, cell viability, chemotaxis, cytotoxicity, and apoptosis.



II. Applications:

- Measurement of cell proliferation in response to growth factors, cytokines, mitogens and nutrients
- Analysis of cytotoxic and cytostatic compounds such as anticancer drugs, toxic agents and other pharmaceuticals
- Assessment of physiological mediators and antibodies that affect cell growth

III. Sample Type:

- Adherent and suspension cells
- Proliferating and non-proliferating cells

IV. Kit Contents:

Components	NBP2-54883	Cap Code
MTT Reagent	50 ml	NM
MTT Solvent	150 ml	WM

V. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom.
- Multi-well spectrophotometer (ELISA reader)

VI. Storage Conditions and Reagent Preparation:

Store kit at - 20°C, protected from light. Centrifuge small vials prior to opening. Read entire protocol before performing the assay.

- **MTT Reagent:** Protect from light. Open under sterile conditions. Thaw at room temperature (RT).
- **MTT Solvent:** Thaw at RT. Use within 2 months.

VII. MTT Cell Proliferation Assay protocol:

1. Grow cells at varying densities (10^6-5x10^6 cells per ml) in a clear plate according to the desired protocol. Dissolve compounds of interest in an appropriate solvent. Treat cells with compound for desired time period. Prepare parallel well(s) as solvent control and use same volume of solvent as for the treated cells. For adherent cells, carefully discard the media. For suspension cells, spin the 96-well plate at 1,000 X g, 4°C for 5 min. in a microplate compatible centrifuge and carefully discard the media. Add 50 µl of serum-free media and 50 µl of MTT Reagent into each well. For background control, add 50 µl of MTT Reagent into a well containing media only (no cells). Incubate the plate at 37°C for 3 hrs. After incubation, add 150 µl of MTT Solvent into each well. Wrap the plate in a foil and shake on an orbital shaker for 15 min. Read absorbance (590 nm).

   Notes:

   a. Cells seeded at densities between 5,000-10,000 cells per well should reach optimal population densities within 48-72 hrs. We recommend using appropriate incubation time depending on the individual cell type and cell concentration used.

   b. Culture conditions such as age of the culture, number of passages, and growth media can affect the result and must be taken into consideration when analyzing the data.

   c. Serum or phenol generates background; prepare parallel well as medium control.

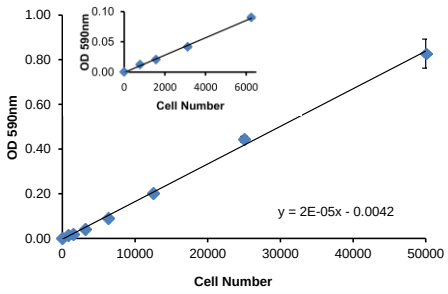


Figure: MTT Cell Proliferation Assay: HeLa cells were grown in DMEM media supplemented with 10% FBS, harvested using trypsin and counted using Trypan blue and a hemocytometer. Cells were serially diluted in a clear cell culture plate and incubated for 3 hrs with MTT Reagent at 37°C. After incubation, cells were treated with MTT Solvent for 15 min. at room temperature. Absorbance was measured at 590 nm. Inset graph is an expanded segment of the assay data at lower cell number per well.