MTT Cell Proliferation and Cytotoxicity Assay Kit

Catalog No.: BRARA156

Kit Component

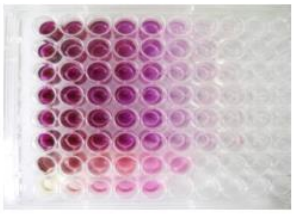
MTT staining solution 5ml

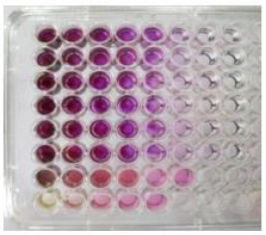
Formazan diluent solution 55ml

Storage: MTT staining solution should be stored at -20℃ in dark; Formazan diluent solution should be stored at room temperature.

Introduction

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 developed by Mossman1 is still among one of the mostversatile and popular assays. The MTT assay involves the conversion of the water soluble MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) to an insoluble formazan.2-4 The formazan is then solubilized, and the concentration determined by optical density at 570 nm. The result is a sensitive assay with excellent linearity up to approximately 96 cells per well.





Protocol

1. Collect logarithmic phase cell, adjust cell suspension concentration; add 100ul floor plate. In general, cells seeded at densities between 1000-10,000 cells per well (side holes filled with aseptic PBS buffer).
2. Seed cells in a 5% CO_2 incubator at 37℃ until cells bespread well bottom for one floor (cells number for each well is according to cells' size and breed speed). Add concentration gradient drug. Add drug after cells adhere. 0-10ul per well. Using 3-5 repeating pipettors.
3. Incubate the cells for 16-48 in a 5% CO_2 incubator at 37℃, then put upside down under microscope to observe.
4. Add 10 μl MTT Reagent to each well, continue to culture for 4 hours. If drug react with MMT, you could centrifugal first then remove nutrient solution. Wash with PBS buffer carefully 2-3 times, add nutrient solution with MTT.
5. Add 100μl Formazan dilution and shake at low speed for 10min until crystal dissolved completely. Measure the absorbance at 570nm using an ELISA reader.
6. Meanwhile, set up blank well（culture media, MTT staining solution, Formanzan solution）, comparation well (cells, drug dissolve media with same concentration, nutrient medium, MTT staining solution, Formanzan solution).

Note

1. Because detecting by 96 wells, if you need culture cells more than 48h or longer, you should better saturate the 64 wells in the middle with sterile PBS buffer. In case that, during culture period, water in side hole evaporates very quickly and nutrient solution or other drug may condense and the cells situation may become complicated.
2. Before use, please preheat MTT solution at room temperature or 20-25℃ for a moment until it is thawing.
3. MTT solution should be storage at 4℃ in dark within 2 weeks. For longer tome storage, you should storage at -20℃, avoid multigelation. Pack it in small package. Once it turn to yellow or green, please stop using it immediately.
4.When Formazan dilution is frozen or appears sediment, you should incubate it at 37℃ water. Before use it, make it is completely melt or well-distributed mix.
5. Incubate it in dark.
6. MTT is oncogenic and sensitive to bacteria, when using it, please wearing gloves and sterilization.