

Protocol for MTT Cell Proliferation Assay

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Description:

MTT assay is a rapid, convenient and safe method (no radioactive) of widely used to measure cell Proliferation by determining viable cell number. It is used in cell apoptosis, cytotoxicity and attachment. The CellTiter 96[®] Assay is based on the cellular conversion of a tetrazolium salt into a formazan product that is easily detected using a 96-well plate reader. The assay is performed by the addition of a premixed optimized Dye Solution to culture wells of a 96-well plate usually containing various concentrations of growth factor or test substance. During 4-hour incubation, living cells convert the tetrazolium component of the Dye Solution into a formazan product. The Solubilization/Stop Solution is then added to the culture wells to solubilize the formazan product, and the absorbance at 570nm is recorded using a 96-well plate reader. The 570nm absorbance reading is directly proportional to the number of cells normally used in proliferation assays.

Experimental Procedure:

1. Plate cells in 50-70% density to a triplicate set of wells with 96-well plate. When cells attach, infect the cells with the proper titer of virus.
2. After 16 hours infected cells, change medium with 0.5% (or 10%) serum medium, incubate 37C, 24-72 hours (according to your experimental design).
3. Add 15 ul Dye Solution (Promega, part#: G4O2A) to each well and mix well, put back to incubate 4hours continuously.
4. Add 100ul/well Solubilization/Stop Solution to stop the reaction and to solubilize the formazan crystals in a humidified atmosphere for overnight (you can choose: incubate 37 C 1hour, the mix using multichannel pipette. Notice: avoid bubble formation, because it can interfere with the accurate recording of absorbance values).
5. Record the absorbance at 570nm wavelength using a 96-well Microplate Reader.

Note:

In the step 4 ,you can aspirate the medium carefully only leave attaching and living cell, then add 100ul/well Solubilization/Stop Solution to solubilize the formazan pure crystals solution, it appear blue, no other interfere color and have accurate concentration(because all of crystals in 100ul). And in control well, on-cell has very low background. I always choose this method.