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XTT Cell Proliferation Assay

Cat. No: XV031CA-F, XV031CA-S, XV031CA

Introduction

The XTT Cell Proliferation Assay kit is a colorimetric assay that detects cellular metabolic activities. During the assay, the yellow tetrazolium salt XTT (sodium 2,3,-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino)-carbonyl]-2H-tetrazolium) is reduced to a highly colored formazan dye by dehydrogenase enzymes in metabolically active cells. This conversion only occurs in viable cells and thus, the amount of the formazan produced is proportional to viable cells in the sample. The formazan dye formed in the assay is soluble in aqueous solution and can be quantified by measuring the absorbance at wavelength 450 nm using a spectrophotometer. An electron coupling reagent, such as PMS (N-Methylphenazonium methyl sulfate), can significantly improve the efficiency of XTT reduction in cells.

Kit Contents

Components	**1000 Reactions**
XTT Reagent	2 x 25 ml
Activation Reagent	1 ml

Storage & Shelf Life

Upon receipt, store kit at -20°C.

Protect XTT Reagent from light.

Kit components are stable for at least 1 year from date of receipt when stored as recommended.

Preparation of XTT Working Solution

Immediately before use, add 100 µl of the Activation Reagent to 5 ml of XTT Reagent. Prepared XTT Working Solution should be added to cells within several minutes.

If sediment is present in the XTT Working Solution, heath the solution to 37°C and swirl gently until a clear solution is obtained.

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Protocol

1. Plate cells into 96-well tissue culture plates at a density of 10^4-10^5 cells/well in 100 µl. The medium may contain up to 10% serum.
2. Carry out desired cell treatments.
3. Culture the cells in a CO_2 incubator at 37°C for 24-48 hours.
4. Add 50 µl of the XTT Working Solution to each well. Mix gently for 1 minute.
5. Incubate the cells for two hours (adherent culture) to four hours (suspension culture) at 37°C in a CO_2 incubator

   Note: *The optimal incubation time for this assay depends on experimental setup, such as: cell type, cell number, and treatment. Optimization of incubation time can be determined by reading one plate at various time points after addition of XTT Working Solution.*
6. Shake the plate gently to evenly distribute the dye in wells.
7. Measure the absorbance signal of the samples with a spectrophotometer at a wavelength of 450-500 nm. Measure background absorbance at a wavelength 630-690 nm. Subtract background absorbance from signal absorbance to obtain normalized absorbance values:

   $$\mathbf{A = A_{450\text{-}500nm}(Test) - A_{450\text{-}500nm}(Blank) - A_{630\text{-}690nm}(Test)}$$
8. Plot the absorbance values of your normalized data on the ordinate (Y-axis) and your experimental parameters (e.g., number of cells) on the abscissa (X-axis).