SERVA
Electrophoresis

PRODUCT INFORMATION

XTT Cell Proliferation Assay Cat. No. 39904

Introduction

XTT Cell Proliferation Assay Kit is a colorimetric assay that detects the 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 coloured 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.

Advantages/Features:

- Easy to use: there is no need for additional reagents and/or cell washing procedures.
- Rapid (no solubilization step as in an MTT assay) results within 2 - 5 hours.
- Sensitivity: Can be assayed even in low cell concentrations.
- Accuracy: Dye absorbance is proportional to the number of cells in each well.
- No radioactivity
- Ideal for high throughput assays (no washing or other steps that can cause cell loss and variability)
- The entire assay can be performed directly in a microtiter plate.

Storage
Upon receipt, store the kit at - 20 °C. Protect XTT solution from light.

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

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

Assay Procedure

1. Plate cells into 96-well tissue culture plates at a density of 10^4 - 10^5 cells per well in 100 µl and the medium may contain up to 10 % (v/v) 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 reconstituted XTT mixture to each well. Mix gently for one 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 solution.

---

6. Shake the plate gently to evenly distribute the dye in the 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 between 630 - 690 nm.

   Subtract background absorbance from signal absorbance to obtain normalized absorbance values:

   $A = A_{450\text{-}500\ nm}$ (Test) – $A_{450\text{-}500\ nm}$ (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).

Version 12/17

SERVA Electrophoresis GmbH • D-69115 Heidelberg • Carl-Benz-Str. 7
Tel.: +49(0)6221 / 138 40-0 • Fax• +49(0)6221 / 138 40-10 • E-Mail:
info@serva.de http://www.serva.de