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XTT CELL PROLIFERATION ASSAY

Cat. No.: CP-001-500 (500 Assays)

CP-001-1000 (1000 Assays)

Store at: -20°C

Instruction Manual

Product Description

The XTT Cell Proliferation Assay Kit provides a rapid and sensitive colorimetric method for the quantitative measurement of cell viability and proliferation in cultured cells. The assay is based on the metabolic reduction of XTT to a soluble formazan product by viable cells.

Principle of the Assay

Metabolically active cells reduce the tetrazolium salt XTT in the presence of an electron-coupling reagent to form an orange-colored, water-soluble formazan dye. The quantity of formazan produced is directly proportional to the number of viable cells and can be measured spectrophotometrically at 450–500 nm. A reference wavelength of 630–690 nm is recommended to correct for nonspecific absorbance.

Kit Components

Component (CP-001-500) (500 Assays)	Size
XTT Reagent (Sterile)	5x5ml
Activation Reagent (Sterile)	1x0.5ml

Component (CP-001-1000) (1000 Assays)	Size
XTT Reagent (Sterile)	10x5ml
Activation Reagent (Sterile)	2x0.5ml

Storage and Stability

- Store all components at –20 °C.
- Kit components are stable for at least 12 months from date of receipt when stored as recommended.
- Protect XTT reagent from light.
- Avoid repeated freeze–thaw cycles.
- If precipitate is observed, warm to 37 °C and gently mix until a clear solution is obtained.

Experimental Procedure

1. Cell Seeding and Culture

Seed cells into a flat-bottom 96-well containing 100 µL/well of cell culture medium (10^4-10^5 cells/well). Incubate the plate at 37 °C in a humidified incubator with 5% CO_2. Depending on the cell type and experimental design, proliferation analysis is typically performed after 24–96 hours. Include wells containing complete culture medium without cells to serve as background controls.

2. Preparation of XTT Reaction Mixture

Immediately prior to use, thaw both the XTT reagent and the activation reagent in a 37 °C water bath. Gently mix by swirling until the solutions are fully dissolved and clear.

For one 96-well plate, prepare the reaction solution by combining 5 mL of XTT reagent with 100 µL of activation reagent. Mix gently to ensure homogeneity.

3. Addition of XTT Reaction Solution

Add 50 µL of the prepared reaction mixture to each well. Incubate the plate at 37 °C for 2–24 hours. In most experimental settings, an incubation period of 2–4 hours are sufficient for adequate color development.

Note: For cells seeded at a ratio of 5-15x10³ cells/well in 80 µl of medium, 40 µl of activation reagent can be added to 4 ml of XTT reagent, and then 40 µl of XTT mixture can be added to each well.

4. Measurement of Absorbance

After incubation, gently agitate the plate to ensure uniform distribution of the colored formazan product within each well.

Measure absorbance using a microplate reader (ELISA reader) at 450–500 nm. To correct for nonspecific background signals, record reference absorbance at 630–690 nm and subtract this value from the primary measurement.

5. Calculation

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

$A = A_{450\text{-}500nm}(Test) – A_{450\text{-}500nm}(Blank) – A_{630\text{-}690nm(}Test)$

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).

Notes

- **For research use only. Not to be used in clinical diagnostic or clinical trials.**
- Defrost and prepare the reaction mixture only immediately prior to use.
- Since the test is extremely sensitive, it is possible to use a low concentration of cells in the wells (approximately 5000 cells per well). Since there are cell types which show low metabolic activity, such as lymphocytes, keratinocytes and melanocytes, it is recommended to increase the concentration of cells to $2.5x10^5$ cells per well, in order to obtain development of formazan color within a reasonable period of time.
- Incubation time with the reaction mixture varies according to the type and concentration of the cells. Therefore, it is advisable to perform an initial test by reading the absorbance at various time lapses, i.e. after 4, 6, 8, 12 hours using the same plate.
- Prior to reading the absorbance with a spectrophotometer, the plate should be gently shaken in order to evenly distribute the dye in the wells.
- If the volume of the media in each well is larger than 100µl, add a larger amount of reaction mixture by the same increment.

Advantages

- There is no requirement for additional reagents and/or the cell washing procedures.
- Multiwell plates and an ELISA reader can be used for reading.
- Can be assayed even in low cell concentrations.
- Dye absorbance is proportional to the number of cells in each well.
- There is no need for radioactive isotopes