ab308239 – XTT Cell Viability Assay Kit (Colorimetric)

Studying cell proliferation and cell cytotoxicity
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: abcam website

Storage and Stability

Store the kit at -20 °C, protected from light. Centrifuge all small vials prior to opening. Read the entire protocol before performing the assay.

Materials Supplied

Item	Quantity	Storage Condition
XTT Reagent (Avoid light)	5 ml	-20°C
Electrocoupling Solution (Avoid light)	100 µl	-20°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Cells and cell culture media
- Cell culture hood and temperature-controlled CO2 incubator
- 96 well clear bottom plate (sterile, cell culture grade)
- Microplate reader capable of measuring absorbance at 475 nm

Reagent Preparation

- For one 96 well plate, add 0.1 ml of the pre-warmed Electrocoupling Solution to 5 ml of the pre-warmed XTT reagent. Mix thoroughly.

Δ Note: *Prepare fresh prior to use. Solution should be clear and not cloudy. If cloudy, warm to 37 °C and mix again.*

Assay Protocol

1. Seed the optimized number of cells in a sterile 96-well, clear bottom tissue-culture microplate. Incubate cells with the desired dose(s) of the test compounds or without any treatment or solvent controls for the desired period of time (usually 20 to 48 hr) at 37 °C in standard cell culture conditions. Include a Background Control well containing only the complete growth media for blank absorbance readings. The final volume of the culture medium in each well should be 100 µl.
2. Prepare the XTT Reagent working solution. Add 50 µl of the XTT working reagent solution to each well. Mix by gently tapping the sides of the plate.
3. Incubate the plate at 37 °C for 0.5-4 hr depending on the cell type and cell density.
4. Read the plate at 30 min intervals by measuring the absorbance at 475 nm until the signal is in the desired absorbance range. Gently tap the plate to ensure that the dye is thoroughly mixed into the solution prior to each reading.

Δ Note:

1. *This assay was developed with HeLa (adherent) and Jurkat (suspension) cells and can be modified for any cell line. Growth conditions, optimal number of cells seeded per well and treatment times should be adjusted based upon the cell line used.*
2. *Appropriate incubation time depends on the individual cell type and cell concentrations used. Therefore, it is recommended to determine the optimal cell counts (usually between 103 and 5x105 cells) and optimal incubation time for your specific cells. Optimization is important because the best assay results are obtained in the linear region of the curve. Cells with low metabolic activity may require higher cell concentrations and/or longer incubation times.*
3. *For toxicity studies, it is recommended to start with more cells. Prepare parallel well(s) as Solvent Control(s) if the test compounds are dissolved in non-aqueous solvent (such as DMSO or acetonitrile).*

Calculation

Subtract the blank absorbance from all the OD 475 nm readings to obtain the normalized absorbance values

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)