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Product Information

Version 1.0.1

A4189– XTT, Sodium Salt

Product Description

XTT is a colorimetric assay used to assess cell viability as a function of cell number based on metabolic activity. This rapid, sensitive, non-radioactive assay is detected using standard microplate absorbance readers. The XTT assay has been reported to be more sensitive than the MTT assay.

XTT is a negatively charged, tetrazolium salt that turns orange when it is reduced to a soluble formazan dye. This extracellular reduction is carried out by electron transport across the plasma membrane of a living cell. Because the reduction occurs by electron transport from outside the cell, the use of an intermediate electron acceptor such as phenazine methosulfate (PMS) is required for complete reduction of XTT. The amount of XTT reduced is reflective of the cellular metabolic activity. The absorbance can be measured and compared to the absorbance of a control solution of untreated cells to determine if cellular metabolic activity has increased or decreased. Thus, XTT can be used to assess cell proliferation or cytotoxicity of drugs.

Cell Viability Assay Protocol

1. Grow cells in a 96-well plate at a density of 104–105 cells/well in 100 µL of culture medium with compounds to be tested. Culture in a CO2 incubator for 24–48 hours.
2. Make a 10 mM PMS solution in phosphate-buffered saline (3 mg PMS into 1 mL PBS).
3. Dissolve 4 mg of XTT in 4 mL of 37°C cell culture medium.
4. Add 10 µL of the PMS solution the 4 mL of XTT solution created in step 3 immediately before labeling cells.
5. Add 25 µL of XTT/PMS solution directly to each well containing 100 µL cell culture.
6. Incubate for 2 hours at 37°C in a CO2 incubator.
7. Read absorbance at 450 nm.

Notes:

- If the absorbance of the blanks is high, the culture medium may contain a reducing agent and an alternative medium should be used.
- A standard curve can be generated to determine optimal cell densities by performing the experiment with a range of known cell densities and measuring the absorbance at 450 nm. The optimal cell density will fall on the most linear part of the plot and have an absorbance below 1.0.

Storage Condition

Stored at -20°C, protect from light.

References

Scudiero, D. A., Shoemaker, R. H., Paull, K. D., Monks, A., Tierney, S., Nofziger, T. H., ... & Boyd, M. R. (1988). Evaluation of a soluble tetrazolium/formazan assay for cell growth and drug sensitivity in culture using human and other tumor cell lines. *Cancer Research*, *48*(17), 4827-4833.

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Berridge, M. V., Herst, P. M., & Tan, A. S. (2005). Tetrazolium dyes as tools in cell biology: new insights into their cellular reduction. *Biotechnology Annual Review*, *11*, 127-152.

**** This product is for laboratory research purpose only. Not for human or animal diagnostic and therapeutic use.***