



MTT Cell Proliferation Assay Kit

Item No. 10009365

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1180 E. Ellsworth Rd • Ann Arbor, MI • USA

Materials Supplied

Kit will arrive packaged as a 4°C kit. This kit will perform as specified if stored as directed and used before the expiration date indicated on the outside of the box.

Item Number	Item	480 wells Quantity/Size	Storage
10009591	MTT Reagent (powder)	1 vial/25 mg	4°C
10009322	Cell-Based Assay Buffer Tablet	1 tablet	RT
600081	Crystal Dissolving SDS	5 vials/1 g	RT
600082	Crystal Dissolving (hydrochloride)	5 vials/10 ml	RT

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

If you have problems, please contact Technical Service:

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Email: techserv@caymanchem.com

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).



WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

Materials Needed But Not Supplied

1. Adjustable pipettes and a repeating pipette
2. A 96-well plate for culturing cells
3. A 96-well plate reader capable of measuring absorbance at 570 nm
4. Distilled water

About This Assay

Cayman's MTT Proliferation Assay Kit provides a convenient tool for studying the induction and inhibition of cell proliferation in any *in vitro* model. In this assay, MTT is taken up by cells due to its net positive charge and the plasma membrane potential. It is reduced to formazan by intracellular NAD(P)H-oxidoreductases.¹

NOTE: This assay can detect 10,000-250,000 cells per well, depending on cell type.

Reagent Preparation

1. Assay Buffer Preparation

Dissolve the cell-based assay buffer tablet (Item No. 10009322) in 100 ml of distilled water. This buffer should be stable for approximately one year at room temperature.

2. MTT Reagent Preparation

Dissolve the entire contents of MTT reagent (Item No. 10009591) in 5 ml of assay buffer by vortexing. The resulting solution should appear bright yellow in color. In the event that the powder does not completely dissolve, remove the undissolved material by filtration or centrifugation. If the entire vial of reconstituted MTT reagent will not be used in a single experiment, we recommend that you aliquot it into smaller sizes and store at -20°C. When stored at -20°C, the reconstituted MTT reagent will be stable for several months. Avoid repeated freeze/thaw.

3. Crystal Dissolving Solution

Immediately before use, dissolve the vial of crystal dissolving SDS (Item No. 600081) with the entire vial of crystal dissolving (hydrochloride) (Item No. 600082). Mix well. Each vial makes a sufficient volume of crystal dissolving solution for a single 96-well plate, using 100 µl/well.

Procedure

1. Seed cells in a 96-well plate at a density of 10,000-250,000 cells per well in 100 µl of culture medium with or without compounds to be tested. Culture the cells in a CO₂ incubator at 37°C for 24-48 hours.
2. Add 10 µl of MTT reagent (prepared above) to each well using a repeating pipettor.
3. Mix gently for one minute on an orbital shaker.
4. Incubate the cells for three to four hours at 37°C in a CO₂ incubator. After incubation, the formazan produced in the cells will appear as dark crystals in the bottom of the wells.
5. Add 100 µl of crystal dissolving solution to each well, and incubate for 4-18 hours in a 37°C CO₂ incubator. Incubation time will vary based on cell densities. This solution will dissolve the formazan crystals, producing a purple solution.
6. Measure the absorbance to each sample at 570 nm using a microplate reader.

Sample Data

An example of typical data obtained with this assay is shown in Figure 1 (below). Your data will vary depending on the cell line and culture conditions used.

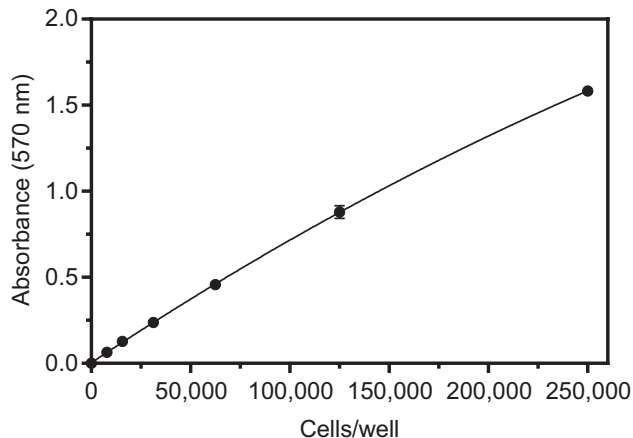


Figure 1. A typical cell titration experiment using HL-60 target cells.

Reference

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

NOTES

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