MTT Assay

Introduction

MTT assays are typically used to measure the **viability** and **proliferation** of cells. They can be commonly applied to study growth of cells under various conditions (e.g., drug treatments or gene modulation conditions). Keep the key limitation in mind – this assay will not work if your drug or condition interferes with reduction of the reagent.

Adapted from: https://www.abcam.com/kits/mtt-assay-protocol

Materials

Lab Machinery	Standard Supplies	Specific Reagents
Fluorescent Plate Reader	96-Well Plate	MTT Reagent
Scale	15-mL Falcon Tube	DMSO
	dPBS	
	Multichannel Pipette	
	Reservoirs	

Preparation

1. Prepare your MTT stock reagent. Note that MTT is soluble in water (10mg/mL), ethanol (20mg/mL), and dPBS or culture media (5mg/mL). We typically using a 5mg/mL solution in dPBS and mix by vortexing.

   Tip: This solution can be stored at 4°C for 3-5 days or at -20°C for approximately 6 months so you do not have to remake it each time you perform the assay.

   a) For 10mL of MTT stock reagent, measure out 50mg (0.05g) of MTT powder into a 15mL falcon tube.
   b) Add 10mL of dPBS and mix thoroughly by vortexing and agitating until the mixture is thoroughly mixed and has no pellets or debris.
   c) Filter sterilize this solution and save it as the **MTT stock reagent.** It should appear bright yellow.

2. Ensure that you have **sterile** dimethyl sulfoxide (DMSO) in the lab for the MTT solvent.

3. Plate your cells **exactly evenly (same # per well) and in replicates (4-8 minimum)** into a 96-well-plate **at least 24 hours in advance** (longer if you have treatment conditions) and ensure they are properly attached and healthy before performing this assay.

   Tip: If treating cells for multiple days, plate them in the 96-well plate at the start of the treatment course at an appropriate density such that they can withstand treatment and grow for the period of the treatment without overgrowing the plate. At the final day of your treatment course, perform the MTT assay.
   Tip: In our experience, approximately 3000-5000 cells per well for moderately rapidly proliferating cells (doubling time ~24-36 hours) was sufficient to allow for 3 days of treatments followed by an MTT assay.

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Protocol

1. Remove your cells from the incubator and check them carefully. The control cells should **not** be overgrown. They should be well attached with minimal debris and appear healthy.

   Tip: If you have a group that you expect to be highly proliferative, check that group as well. It is possible in some cases for the cells to overgrow and detach, thus resulting in a falsely decreased result with this assay. In this scenario, you will have to replate the cells with lower density per well and repeat the assay.

2. Make your **MTT working solution** by diluting your MTT stock solution 1:10 in the cell culture media, making 110uL of working solution for each well you want to assay. Place this solution into a **sterile** reservoir.

   *Tip: Calculate and make enough for all the plates you want to assay (and ideally a little bit extra). For example, if we were assaying one 96-well plate, we would calculate for 100 wells. For 100 wells, at 110 uL per well, we would need to make 11mL of **MTT working solution.** To do this, we would add 10mL of culture media and 1mL of **MTT stock solution** into a 15mL tube. Mix well and place into a **sterile** reservoir.*

3. In the tissue culture hood, remove the media from the plate.

   Tip: The best way to do this is to use a multichannel to completely remove the media from each well, working only from the corners of the wells to avoid dislodging cells and pipetting up and down 2-3 times to get rid of any debris. Wash the cells 1-2 times with sterile dPBS in the same volume used for culture media (~100-200 uL), removing completely each time.

4. Once the cells are washed, remove the final dPBS from the plate completely. Add in the MTT working solution (i.e., MTT + media solution), using 110uL per well and dispensing with a multi-channel from a reservoir.

5. Check the plate under the microscope to ensure cells are still attached. They should still appear healthy at this point although the media may seem slightly more orange. Place the plate back into the 37 degree incubator and incubate for 3-5 hours.

   Tip: This can be left 24 hours if necessary

6. After the time period has completed, remove the plate from the incubator and check the plate under the microscope. You should note formation of crystals on the base of the plate and the cells should be gone. These are the formazan crystals you will be quantifying.

7. **CAREFULLY** remove the media, pipetting only from the corners, and using **only** a multi-channel pipette to remove the media. Remove completely – any residual media will affect the precision of the assay moving forward.

8. Resuspend the cells in 100uL of DMSO per well, pipetting aggressively up and down to resuspend the crystals completely. You should see the wells turn a deep purple in the most proliferative cells that you noted on your initial examination of the plate. Allow the plate to sit for 10 minutes (or up to 1 hour) before reading it out. Some gentle agitation on a plate rocker can help during this time.

9. Read out the plate at OD=590 to get the absorbance values and save them into an excel sheet.

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Analysis & Sample Results

Analysis is highly dependent on your assay question. For detailed information on analysis and on ensuring your initial plate design is appropriate, please reference the videos at www.benchbytes.com. However, sample instructions follow for the common use case of dose response:

For a Drug Response Curve:

Conc	0 uM	1 uM	2 uM	5 uM	10 uM	20 uM	50 uM	100 uM	200 uM	500 uM	1000 uM	DMSO ctrl
Rep 1	1.0345	0.8566	0.7498	0.6817	0.5363	0.4573	0.3923	0.33492	0.2859	0.2293	0.1839	0.1323
Rep 2	1.014	0.809	0.7618	0.6965	0.5317	0.4673	0.3859	0.3295	0.2893	0.2339	0.1834	0.1232
Rep 3	1.283	0.8248	0.7943	0.6462	0.5957	0.4294	0.3895	0.3129	0.2793	0.2321	0.1834	0.1323
Rep 4	0.792	0.8815	0.7535	0.6411	0.5673	0.4395	0.382	0.3294	0.2953	0.2134	0.1734	0.1432
Rep 5	1.282	0.8094	0.7436	0.6046	0.5782	0.4683	0.3958	0.3352	0.2893	0.2212	0.1983	0.1395
Rep 6	0.717	0.9023	0.7685	0.668	0.5789	0.4592	0.3958	0.3395	0.271	0.2234	0.1893	0.1295
Rep 7	0.698	0.8112	0.7445	0.6383	0.5334	0.4293	0.3923	0.3294	0.2893	0.2354	0.1793	0.1234
Rep 8	0.994	0.8159	0.7776	0.6845	0.5638	0.4538	0.3972	0.3123	0.2931	0.2343	0.1934	0.1234
Average	0.9768125	0.8388375	0.7617	0.6576125	0.5606625	0.4505125	0.39135	0.32789	0.2865625	0.227875	0.18555	0.13085

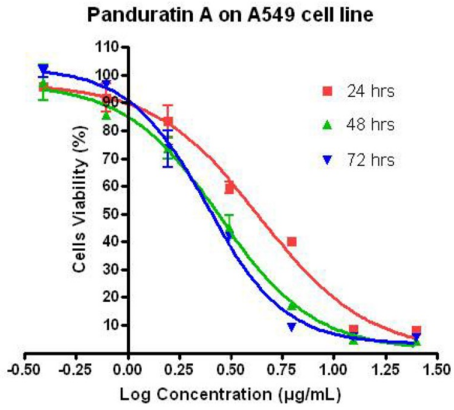
To calculate the normalized % viability for each absorbance, using Rep 1 at 0 uM as an example, use the following formula:

$((Abs_{Rep1,\ 0uM} - Abs_{DMSO\_ctrl\_avg})/(Abs_{Avg,\ 0uM} - Abs_{DMSO\_ctrl\_avg}))*100$

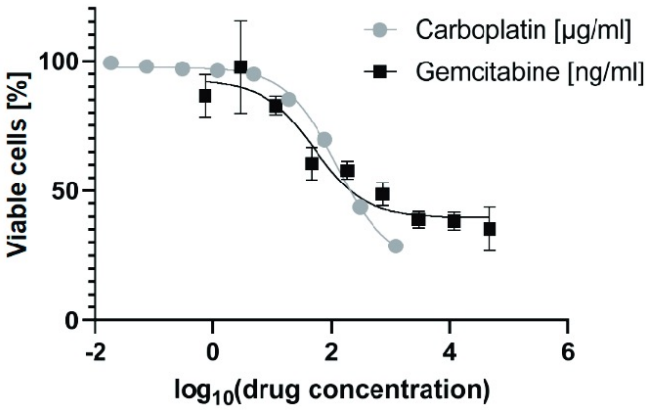
As you move across the columns, replace the 0uM average with the appropriate average for the dose you are on. As a control (so you can test your excel formula), this should give you 106.8192% viability for Rep 1 0uM and 30.905% viability for 50uM Rep 1.

Enter these values into GraphPad prism to get a final dose response curve.

Examples of good data are below with their attributions:



Cheah et al. Dec 2011. Article.



Bjorn et al. May 2020. Article.