Method Sheet 08

MTT assay for mammalian cell metabolic activity

Overview

This method sheet explains how to perform a basic version of the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay (MTT) assay. This method is commonly used to measure the metabolic activity of cells as a proxy for the effects of drugs on cell viability or proliferation. Metabolically active (viable) cells express enzymes that reduce the soluble, yellow tetrazolium salt MTT to insoluble formazan crystals, which are purple in colour. The intensity of the purple color, which is measured spectrophotometrically at ~570 nm, is broadly proportional to the number of viable cells. However, note that formazan synthesis may be affected not only by changes to cell viability, but also by changes to cellular metabolic activity or rate of proliferation. Furthermore, some natural compounds are capable of reducing MTT to formazan in the absence of cells, and some plant pigments can interfere with the absorbance measurements. Thus, some caution is necessary in the interpretation of results from this assay, as it is possible to observe both false negative and false positive results with respect to cell viability (see notes section, below).

Reagents

- MTT reagent (5 mg/ml in PBS, see notes below on how to prepare)
- 10% SDS solution (store at room temperature)

Equipment

- Microplate reader capable of measuring absorbance at ~570 nm (any filter between 550 nm and 600 nm will be suitable)
- Multi-channel pipette capable of dispensing 25 - 100 µl

Method

1) Challenge cells with drug of interest then incubate at 37°C for 24-72 hours (see Method Sheet 07).
2) This assay requires a positive control for inducers of cell death, which can be achieved by pipetting 10 µl of 10% SDS solution into each well of column 12 (this kills all the cells in this column, which can then serve as a baseline for 100% cell killing).
3) Be careful not to accidentally splash SDS solution into any other wells of the plate while doing this.
4) Remove one MTT stock reagent vial from the fridge and aliquot 3 ml of this stock per microplate into a sterile dispensing reservoir, using sterile technique in the biosafety cabinet.
5) Return any unused MTT reagent to the fridge for later use before continuing.
6) Adjust a multichannel pipette to dispense 25 µl.
7) Firmly press the pipette into a row of sterile pipette tips (8 or 12, depending on the type of pipette), ensuring all are securely attached.

8) If the tips are loose or fall off during use, use gloved finger and thumb to pull up and seat firmly each tip individually, being very careful to touch only the upper part of the tip, and not the lower part of the tip which must remain sterile before use.
9) Push the plunger on the pipette down to the first stop (not all the way to the second stop).
10) Insert the tips into the liquid in the reservoir, ensuring all tips are below the surface of the liquid.
11) Slowly release your thumb to allow the plunger to return to the top position.
12) Look carefully across all the tips to ensure the level of liquid is the same in each tip, if not, dispense the suspension back into the reservoir and try again.
13) Likewise, if there are any large air bubbles in any of the tips, dispense back into the reservoir and try again.
14) Move the pipette to the open sterile plate and dispense all the liquid into the wells of the next available column, pipetting past the first stop all the way to the second stop of the plunger this time.
15) Repeat this process until every well in the plate has received MTT reagent.
16) Gently move the plate backwards and forwards to mix.
17) Incubate the plate at 37°C for 4 hours (the assay will also work, albeit with lower signal, at 3 hours, but keep the duration of incubation the same for each experiment).
18) Aliquot 12 ml of 10% SDS solution per microplate into a pipetting reservoir.
19) Use a multichannel pipette to add 100 µl of 10% SDS solution to all wells in the plate, including the positive and negative controls.
20) Gently move the plate backwards and forwards several times to mix.
21) Incubate the plate in a dark place (e.g. a cupboard) overnight at room temperature.
22) At any time the next day, measure the absorbance of every well in the microplate at a wavelength of 570 nm, or alternatively anywhere between 550 and 600 nm if not available, using a microplate reader.
23) There is no requirement for use of a reference wavelength, but if one is prefered, a wavelength of 670 nm or above can be used.
24) Retrieve the data from the microplate reader software for further analysis using Microsoft Excel (see Method Sheet 17).

Notes

- The protocol assumes that the user is trained in the basic principles and techniques of mammalian cell culture - please seek such training if you have not received it already before attempting to follow the method.
- If the SDS solution appears cloudy, simply place in a 37ºC incubator or water bath to fully dissolve until clear before use.
- MTT reagent (also called 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) can be purchased inexpensively as a pure compound from most life science suppliers.

- To prepare the 5 mg/ml stock of MTT reagent, add 100 mg MTT powder to 20 ml PBS (or multiples thereof), mix by inversion until fully dissolved, then filter sterilise using a plastic syringe attached to a 0.44 µm filter and aliquot into foil-wrapped tubes.
- Store the MTT stock solution in foil-wrapped tubes in the fridge (i.e. at around 4ºC) for up to several weeks before use.
- Some test extracts or compounds can absorb at the same wavelength as MTT reagent, or chemically reduce it, giving rise to false positive increases in readings. In these instances repeat the assay with the extract or compound and MTT reagent in medium alone without cells. If there is an increase in absorbance in this case, this value should be subtracted from the measurements of the cellular assay, or an alternative assay of cell viability not prone to such interference should be used (such as the crystal violet assay).