MTT Assay Protocol	
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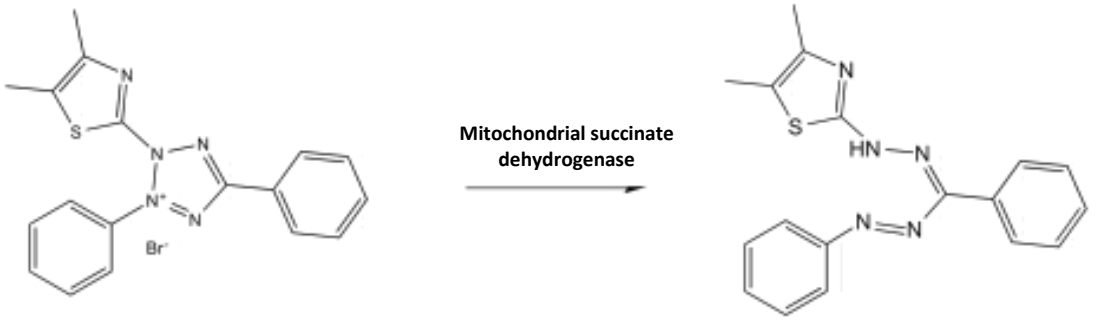
Approved by: Dr. Susu Zughaier	**Protocol Number**: P003

Background:

MTT assay is an assay that allows researchers to assess cellular metabolic activity, and hence allow for observation of cellular viability. The enzyme succinate dehydrogenase (present in the mitochondria) is able to reduce the yellow soluble tetrazolium dye MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide to an insoluble form, formazan which has a purple color. Hereafter, an organic solvent is used to solubilize formazan and allow the dye to be released. The intensity of the dye is measured spectrophotometrically at 591 nm which reflects the viability of the cells.



3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide **(MTT)**

(*E*,*Z*)-5-(4,5-dimethylthiazol-2-yl)-1,3-diphenylformazan **(Formazan)**

Reagents:

MTT Reagent (yellow powder)
DMSO
PBS – Sterile
Treated/untreated cells

Materials:
Balance
Pipette Gun
Multi-Channel Pipette
Pipettes of different sizes (10 mL, 25 mL, 1000 uL, 200 uL, 20 uL)
50 mL conical tube.

Procedure:

a) Reagent Preparation:
   1. To prepare MTT reagent you need to dissolve 5 mg of MTT reagent in 1 mL of PBS (You will probably need more than 1 mL, so do the calculation for it. E.g. if you need 5 mL of PBS then weigh 25 mg of MTT reagent and dissolve in 5 mL of

PBS). NOTE: ALWAYS WEAR GLOVES AND BE CAREFUL WHEN HANDLING MTT REAGENT.

b) MTT Assay:

1. Either collect soaps from cells if required, or discard them from the cells.
2. Wash cells 1x with PBS to remove residual media.
3. Add 150 uL of fresh media to the cells.
4. Add 15 uL of MTT reagent to each well.
5. Incubate the cells at 37 ^{o}C for 2 hours.
6. After incubation, remove media with MTT reagent, and wash cells with PBS 1x to remove residual media
7. Remove PBS Add 150 uL of DMSO to the cells
8. Keep the cells on the shaker for 5 minutes, or on the settings on TECAN machine, choose shaking for 5 minutes before reading out the result.
9. Measure absorbance using TECAN (or any other spectrophotometer) at 591 nm.

Storage Conditions:

Reagent	Storage
MTT Reagent	4 ^{o}C
DMSO	25 ^{o}C

Hazards:

Reagent	Hazard
PBS	None
DMSO 	1. Combustible liquid. 2. Causes skin irritation. 3. Causes serious eye irritation.
MTT Reagent 	1. Causes skin irritation. 2. Causes serious eye irritation. 3. May cause respiratory irritation. 4. Suspected of causing genetic defects.