MTT ASSAY

Assays which allow for the quantitative measurement of cell death during cell culture are crucial to any experiment involving cell lines or ex vivo cellular clinical samples. The MTT assay is used to measure cellular metabolic activity as an indicator of cell viability, proliferation and cytotoxicity. This colorimetric assay is based on the reduction of a yellow tetrazolium salt (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide or MTT) to purple formazan crystals by metabolically active cells. The viable cells contain NAD(P)H-dependent oxidoreductase enzymes which reduce the MTT to formazan.

Equipment and materials:

MTT (2-8 °C)

DMSO (Dimethyl sulfoxide)

Detergent reagent (18-24 °C)

96-Well plate (flat bottom)

Incubator

Multi-channel pipette

Serological pipettes

Sterile tubes

Sterile pipettes tips

Laminar flow hood

Inverted microscope

*(Preparation Instructions MTT is soluble in water (10 mg/ml), ethanol (20 mg/ml) and is also soluble in buffered salt solutions and culture media (5 mg/ml). Reconstituted MTT solution is stable for at least 6 months when stored at -0oC. Storage at 4oC for more than four days will result in decomposition and will yield erroneous results)

Safety:

See the Material Safety Data Sheet (MSDS) regarding safety precautions for this method.

Procedure:

Short 96 well assay: EACH condition should be done in triplicate or more.

DAY ONE:

1. Trypsinize one T-25 flask and add 5 ml of complete media to trypsinized cells. Centrifuge in a sterile 15 ml falcon tube at 500 rpm in the swinging bucked rotor (~400 x g) for 5 min.
2. Remove media and resuspend cells to 1.0 ml with complete media
3. Count and record cells per ml. Remember to remove the cells aseptically when counting.
4. DILUTE the cells (cv=cv) to 75,000 cells per ml. Use complete media to dilute cells.
5. Add 100 µl of cells (7500 total cells) into each well and incubate overnight.

DAY TWO:

6.Treat cells on day two with agonist, inhibitor or drug.

- If removing media, do very carefully. This is where most variation in data may occur.
- Final volume should be 100 µl per well.

DAY THREE:

7.Add 20 µl of 5 mg/ml MTT to each well. Include one set of wells with MTT but no cells (control). All should be done aseptically.

8. Incubate for 3.5 hours at 37oC in culture hood.

9. CAREFULLY Remove media. Do not disturb cells and do not rinse with PBS.

10. Add 150 µl DMSO and Sorenson's buffer (0.1 M Glycine, 0.1 M sodium chloride, PH=10.5) into each well to dissolve the formazan.

11. Cover with tinfoil and agitate cells on orbital shaker for 15 min.

12. Read absorbance at 590 nm. The reference wavelength should be higher than 650 nm. The blanks should give values close to zero (+/- 0.1).]. (Spectrophotometric plate reader, ELx800, Biotek)

13. If the readings are low return the plate to the dark for longer incubation.

Interpreting results:

The absorbance reading of the blank must be subtracted from all samples. Absorbance readings from test samples must then be divided by those of the control and multiplied by 100 to give percentage cell viability or proliferation (see formula below). Absorbance values greater than the control indicate cell proliferation, while lower values suggest cell death or inhibition of proliferation.

$$\% \text{ viable cells} = \frac{(abs_{sample} - abs_{blank})}{(abs_{control} - abs_{blank})} \times 100$$

Troubleshooting Problem:

MTT Reagent is blue-green:

cause	Remedy
Contamination with a reducing agent or cell/ bacterial contamination	Discard. Remove aliquots of new MTT Reagent, using sterile technique
Excessive exposure to light	(Store solution in the dark at 4°C)

Absorbance readings too high:

cause	Remedy
Cell number per well too high.	Decrease cell density at plating
Contamination of culture with bacteria or yeast	Discard. View wells prior to addition of MTT Reagent to check for contamination

Absorbance readings are too low:

cause	Remedy
Cell number per well is too low	Increase cell density at plating
Incubation time for reduction of MTT is too short. No purple color visible in cells when viewed under microscope	Increase incubation time with Detergent Reagent until purple color is evident inside cells when viewed under microscope. Longer incubation of up to 24 hours may be required .for some cell types
Incubation time for solubilization of formazan dye too short (intact cells with intracellular dye .visible when viewed under the microscope)	Increase incubation time with Reagent or incubate at 37°C. View under microscope to ensure no crystals remain out of solution

Cells not proliferating due to improper culture conditions or inadequate time of recovery after platin	Check that culture conditions (medium, temperature, humidity, CO2, etc.) are appropriate. View cells periodically to check condition. Increase time in culture after plating .for cell recovery

Replicates have different values:

cause	Remedy
Inaccurate plating or pipetting	Increase accuracy of cell plating, check accuracy of pipette
Contamination of culture with bacteria or yeast	Discard. View wells prior to addition of MTT Reagent to check for contamination

References:

1.PROVOST&WALLERT RESEARCH, Investigating the Biochemistry & of NHV1 EST.1998.van de Loosdrecht, A.A., et al. J. Immunol. Methods 174: 311-320, 1994.

2.MTT Cell Proliferation Assay, ATCC.

3.Cell Viability and Proliferation, Sigma Aldrich.

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دانشگاه علوم پزشکی تبریز

شناسنامه سند: CoreLab.103

نام سند	پروتکل استاندارد MTT ASSAY
تاریخ صدور	۱۳۹۹/۶/۲۹
نام کامل فایل	پروتکل استاندارد MTT ASSAY
شرح سند	روش انجام MTT ASSAY را شرح می دهد.
تهیه کننده	شهربانو نادری