DB-ALM Protocol n° 17 : MTT Assay

Basal Cytotoxicity

This protocol provides a generic description of a simple assay, which can be used to determine the viability/number of cells in culture. The qunatitative measurement is made through a formation of a coloured product (in a mitochondria-dependent reaction) to which the cell membrane is impermeable.

Résumé

The tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)- 2,5-diphenyltetrazolium bromide (MTT) is taken up into cells and reduced in a mitochondria-dependent reaction to yield a formazan product. The product accumulates within the cell, due to the fact that it cannot pass through the plasma membrane. On solubilisation of the cells, the product is liberated and can readily be detected and quantified by a simple colorimetric method.
The ability of cells to reduce MTT provides an indication of mitochondrial integrity and activity which, in turn, may be interpreted as a measure of viability and/or cell number. The assay has therefore been adapted for use with cultures of exponentially growing cells. Determination of their ability to reduce MTT to the formazan product after exposure to test compounds, compared to the control situation, enables the relative toxicity of test chemicals to be assessed.

Experimental Description

Endpoint and Endpoint Measurement:

TISSUE VIABILITY: determined by a reduction in mitochondrial dehydrogenase activity, measured by formazan salt production from MTT. It is expressed as % of the negative control

Endpoint Value:

Tissue Viability (%): calculated as ratio $(OD_{treated})/(OD_{negative\ control})\times 100\%$

IC_{50}: the concentration of a test substance that decreases the MTT reduction to formazan by 50%, determined fom a dose-reponse curve.

Experimental System(s):

CELL LINE CULTURES - the MTT assy can be applied to many cell cultures, provided that the mitochondrial activity is of a reasonably high level.

Basic Procedure

Exponentially growing cells are plated into 96-well microplates and incubated in the absence and presence of test compounds for set periods. The cells are then exposed to MTT for a short time, after which all medium is removed and DMSO added to each well. The amount of solubilised formazan product is determined using a spectrophotometer. That present in test conditions is expressed as a percentage of that occurring in the control situation. Results may be expressed as percent cell survival.

Discussion

The assay is suited to cells which tend to clump as well as those which fail to form colonies, but not to static cell populations nor those with low mitochondrial activity. It may thus be applied to a variety of cell lines provided that they have an exponential rate of growth in culture and that mitochondrial activity is of a reasonably high level. It should be noted, however, that the number of cells initially plated, period of exposure to chemicals, concentration of MTT, total duration of the experiment, etc. must be standardised for each cell line.

A variety of compounds may be tested in this system, provided they can be dissolved in the culture medium. Certain compounds, however, may selectively affect the mitochondria of the cells, resulting in a greatly overestimated level of toxicity. Some chemicals may exert a positive effect upon cell growth. Such a response would be readily detected using this assay.

The assay is relatively simple to perform, can be semi-automated, is rapid, sensitive and adaptable. Its sensitivity means that fewer cells and, therefore, less consumables are required, compared to many test

systems. Thus, it provides a relatively inexpensive method to screen a large number of compounds over a wide range of concentrations.

Formation of the formazan product has been found to correlate well with cell number. The assay has been found to compare favourably with several other methods used to determine cell number/viability in cytotoxicity tests, e.g. clonogenic and dye exclusion tests.

Addendum: Optimisation of the MTT Assay

It is very difficult to outline a standard method for performing the MTT assay applicable to all cell lines. For this reason it may appear that the MTT protocol deposited in the DB-ALM is fairly general. A review of the literature concerning the use of MTT clearly illustrates that there are fairly diverse views on incubation times and especially which solvent should be used to solubilise the formazan crystals. In addition, the MTT assay has various applications, for example, cytotoxicity, evaluation of drug sensitivity and lymphokine secretion, to name but a few.

One of the major problems with the assay is obtaining optical density readings high enough to allow appropriate evaluation at the end of the assay. To optimise cell number/incubation time with the chemical it is suggested that plating out different cell concentrations and incubating them over various time points. In each case the amount of MTT added should be kept constant at 1mg/ml (a concentration used generally by most laboratories). The cells plus substrate should be incubated for a period of 4 hours which is sufficient to produce crystal formation (increasing the incubation period does not appear to noticeably increase the final OD readings although this may vary between cell lines). Care should be taken when removing the medium from the cells because it is easy to dislodge the crystals especially if an aspirator is used. The final question which should be asked is which solvent should be used to solubilise the crystals. The method was originally designed to replace the use of titrated thymidine in suspension cultures (especially cells of lymphoid origin) and, therefore, was designed to allow the development of colour without removal of medium. This, however, was not without problems because of protein precipitation on addition of organic solvent. As a results there are numerous different methods for solubilising the crystals. DMSO is frequently used for adherent cultures although it is not without its problems because of colour changes in the resulting solution due to phenol red from the residual medium which may remain in the well (Bagge Hansen *et al.,* 1989; Tada *et al*., 1986; Denizot & Lang 1986)

Other methods of possible interest:
Landgren, U. (1984)
Dotsika, E.N. & Sanderson, C.J. (1987)

Status

Known Laboratory Use:

National Institute of Health, Betheseda, USA
Istituto Nazionale per lo Studio e la Cura dei Tumori, Milan, Italy

Last update: April 1990

PROCEDURE DETAILS, *April 1990*

MTT Assay
DB-ALM Protocol n° 17

The procedure presented is intended to provide a guide for the performance of the MTT assay with any suitable cell line. General maintenance of cells, seeding density, manner and time of exposure to test compounds, etc., have not, therefore, been stipulated as these will have to be established for individual cell lines. It is hoped, however, that the basic information presented will assist anyone attempting the technique and will be sufficient to help them adapt the assay for their particular needs.

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Materials and Preparations

Cell or Test System

Exponentially growing cell line with recognisable levels of mitochondrial activity.

Equipment

Fixed Equipment

Scanning multiwell spectrophotometer - Easy Reader 400 AT, SLT, Lab Instruments, Salzburg, Austria (or equivalent)
Automatic plate shaker

Consumables

96-well tissue culture plates - Costar, Cambridge, Mass., USA

Media, Reagents, Sera, others

a. Culture medium - dependent on the cell line
b. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) - Sigma
c. Dimethylsulphoxide (DMSO) - Sigma Chemical Co., St Louis, M.O., USA
d. KCl
e. KH_2PO_4
f. NaCl
g. $NaHCO_3$
h. $Na_2PO_4.7H_2O$

Preparations

Media and Endpoint Assay Solutions

a. Hank's salt solution, composition (in g/l): KCl (0.4), KH_2PO_4 (0.06), NaCl (8), $NaHCO_3$ (0.35), $Na_2PO_4.7H_2O$ (0.09)
b. MTT solution: 5mg/ml in Hank's salt solution
N.B. MTT solution may be stored at 4^0C for about one week.

Method

Test System Procurement

Dilute cells to the required concentration in complete medium.
Seed 100µl of cell suspension into 95 wells of a 96-well tissue culture plate.
Add 100µl of culture medium alone to the remaining well (blank).

Test Material Exposure Procedures

(this step may vary according to cell type):

i. Expose cells in suspension to the appropriate concentration of test compound for a short period of time (~ 1 hour), rinse with fresh medium, dilute to the required number of cells/ml and plate out as described above. Incubate the cells for the required time.

ii. Dilute cells in medium containing the appropriate concentration of test compound and plate out as described above. Incubate for the required time.

iii. Dilute cells in medium and plate out as described above, leave the cells to incubate for a set period. Replace the culture medium with complete medium containing the appropriate concentration of test compound and continue to incubate for the required time.

Whichever pattern of exposure is selected set up 8 wells for each concentration of test chemical and run eight wells as controls (no compound present).

Endpoint Measurement

After incubating as required, add 10µl of MTT solution to each well.
Incubate for a further period of time.
At the end of the incubation remove the medium and replace with 100µl DMSO (at room temperature).
Place the plates on a shaker and agitate for 5 mins.
Read the plates at 550nm and 620nm as reference on a scanning multiwell spectrophotometer.

Data Analysis

Record the optical density of each well and correct with reference to the blank.
Percentage cell survival is expressed as:

[(absorbance of treated cells)/(absorbance of control cells)]x 100 %

All results should be the mean of at least 8 determinants.

Dose response curves can be calculated for chemicals tested over a range of concentrations, enabling IC_{50} values to be obtained (i.e. concentration of chemical resulting in a 50% inhibition of cell growth or reducing cell survival by 50%).

Bibliography

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