MTT Assay Kit
For research use only

Catalogue number: BI - 2004

Product Description

A sensitive kit for the measurement of cell proliferation based upon the reduction of MTT. The reduction of MTT to an insoluble colored formazan is primarily due to the glycolytic activity within the cell and is dependent on the presence of NADH and NADPH (thus associated with the mitochondrial metabolic activity).
In actively proliferating cells, an increase in MTT conversion is spectrophotometrically quantified. Comparison of this value to an untreated control provides a relative increase in cellular proliferative activity caused i.e. by trophic factors, growth inhibitors, or inducers/ inhibitors of apoptosis, which may be quantified. Conversely, in cells undergoing apoptosis, MTT reduction decreases, reflecting the loss of cell viability.

Ingredients

1. One vial containing 25mg of MT powder.
2. One bottle containing 30mL of DMSO solution as solvent.
3. One Syringe filter
4. Five amber micro tubes
5. Ready-To-Use without phenol red RPMI 640 medium (100mL).

Kit solution preparation

1. Prepare a 12 mM MTT stock solution by adding 5 mL of Ready-To-Use RPMI1640 (included in the kit) to the 25 mg vial of MTT.
2. Mix by vortexing or sonication until dissolved.
3. Remove particulates by filtration or centrifugation.
4. Equally aliquote the prepared solution in 5 amber vials.
5. Each vial of MTT provides sufficient reagent for 100 tests, using 10 µL of the stock solution per well.
6. Once prepared, the MTT work solution can be stored for four weeks at 4°C, protected from light. Store the remaining stocks at -20°C.

How to use (recommendation)

Some drugs and extracts from plants, may interfere with MTT assay. Before starting the experimentation, make sure that the sample does not contain any material interfering with MTT assay substances.

Determination of optimal Cell Count

This should be only performed once for each cell type.

Cell seeding

1 Culture the cells up to ~ 80% of confluency in a cell culture flask or plate.
NOTE: Use low passage numbers to achieve better results.
2 Harvest suspension cells by centrifugation (5min at 200 x g). Adherent cells should be released from their substrate by trypsinization or scraping. Then, pellet cells by centrifugation at 500 x g for 5 minutes at 2 to 8 °C and discard supernatant.
3 Wash cells by resuspending in 5 mL sterile PBS or cell culture medium. Pellet cells by centrifugation at 500 x g for 5 minutes at 2 to 8 °C and discard supernatant.
4 Resuspend cells at 5 x 10^6cells/mL in culture medium. Harvest and wash sufficient cells to prepare 8 - 10 serial two-fold dilutions with 100 µL of cells/well, in triplicate.
5 Serially dilute cells using 5 mL culture tubes. Dilutions from 5 x 10^6 to 5 x 10^3 cells/mL must be sufficient for most cell types. For example, ten 2-fold dilutions from 5 x 10^6 cells/mL will result in concentrations from 2.5 x 10^6 to 4.88 x 10^3 cells/mL (see table below). For this example, you will need 0.8 mL of cells at 5 x 10^6 cells/mL for a total of 4 x 10^6 cells. Refer to the table.

	Label Tubes (cells/mL)	Add Cell Culture Medium	Add Cells
1	5.00 x 10^6	——	400 µL of 5.00 x 10^6 cells/mL stock
2	2.50 x 10^6	100 µL	400 µL of 5.00 x 10^6 cells/mL stock
3	1.25 x 10^6	100 µL	400 µL of 2.50 x 10^6 cells/mL stock
4	6.25 x 10^5	100 µL	400 µL of 1.25 x 10^6 cells/mL stock
5	3.13 x 10^5	100 µL	400 µL of 6.25 x 10^5 cells/mL stock
6	1.56 x 10^5	100 µL	400 µL of 3.13 x 10^5 cells/mL stock
7	7.81 x 10^4	100 µL	400 µL of 1.56 x 10^5 cells/mL stock
8	3.91 x 10^4	100 µL	400 µL of 7.81 x 10^4 cells/mL stock
9	1.95 x 10^4	100 µL	400 µL of 3.91 x 10^4 cells/mL stock
10	9.77 x 10^3	100 µL	400 µL of 1.95 x 10^4 cells/mL stock
11	4.88 x 10^3	100 µL	400 µL of 9.77 x 10^3 cells/mL stock
12	Medium Control	100 µL	——

6. Plate cells at 100 µL/well. Include 3 control wells of cell culture medium alone.
NOTE: In order to minimize edge effect, we recommend to fill first and last wells with culture media to maintain humidity for the first and last treated wells.
NOTE: Use round-bottomed 96-well plates for non-adherent cells and flat-bottomed 96 well plate for adherent cells.
7. Incubate the cells for 6 - 48 hours. Cells need time to recover and reattach (if adherent). This will vary for each cell type. In general, 12 - 18 hours is sufficient.

MTT staining

1. Add 10 µL of MTT reagent to each well.
2. Incubate the plate for 2 - 4 hours at 37° C. View the cells periodically for the appearance of punctate, intracellular precipitate using an inverted microscope. Some cell types may require a longer incubation, as much as 24 hours.
3. When purple precipitate is clearly visible under the microscope, add 100 µL of Detergent Reagent to all wells (DMSO),
4. Leave covered plate in the dark at 18 - 24° C for at least 2 hours to overnight. Samples may be read after 2 hours, but if the readings are low and there are crystals remaining, return the plate to the dark and incubate for a longer period. Room temperature (18 - 24° C) incubation is sufficient, but incubation at 37° C may shorten the solubilization time.

Data collection, calculation, and interpretation

1. Remove the plate cover and measure the absorbance of the wells, including the blanks, at 570 nm with a reference wavelength of 650 nm. If a 570 nm filter is not available, absorbance may be read with any filter in the wavelength range of 550 to 600 nm. The blanks should give values of 0 ± 0.1 O.D. units.
2. Determine the average values from triplicate readings and subtract the average value for the blank. Plot absorbance on the y-axis versus cell number per mL on the x-axis. Select a cell number that yields an absorbance of 0.75 to 1.25. The cell number selected should fall within the linear portion of the curve

MTT Assay for Experimental Samples Cell seeding

1. Plate cells at the optima concentration determined in the former step. Plate triplicate wells at 100 µL/well for each variable. Be sure to plate enough wells to include cell-based controls, and include three wells of cell culture medium alone.
   NOTE: In general, cells seeded at densities between 5000- 10,000 cells per well should reach optimal population densities within 48- 72 hours.
2. Incubate the cells to allow them to recover and reattach (if adherent) and treat according to your established experimental protocol.

Treatment

1. For adherent cells, remove the medium, wash with dPBS (no calcium, no magnesium), and replace it with Ready-To-Use RPMI1640 (included in the Kit). For non-adherent cells, centrifuge the microplate, pellet the cells, carefully remove as much medium as possible, and replace it with 100 µL of Ready-To-Use RPMI1640.
   NOTE: The presence of phenol red in the final assay can seriously affect results. We strongly recommend that the cells are cultured in a medium free of phenol red (included in the Kit), if possible.
2. Treat cells with various concentrations of the desired compound for 24 hours at 37°C in CO_2 incubator.

MTT staining

1. Add 10 µL of MTT reagent to each well, including controls.
   NOTE: If more than 100 µl of medium is used per well, increase the amount of MTT Reagent accordingly; e.g., for 250 µl of medium use 25 µl of MTT Reagent.
   NOTE: Alternatively, the final incubation with the MTT can be performed after exchanging the cells into the phenol red- free medium.
   NOTE: Prepare a negative control (10 µL of MTT stock solution added to 100 µL of medium alone).
2. Incubate the plate for approximately 2 - 4 hours at 37° C. View the cells periodically for the appearance of punctate, intracellular precipitate using an inverted microscope. Longer incubation times (up to 24 hours) may be required, depending on the cell type and experimental conditions. At high cell densities (> 100,000 cells per well), the incubation time can be shortened to 2 hours.

3. When purple precipitate is clearly visible under the microscope, add 100 µL of Detergent Reagent to all wells, including control wells. Do not need to remove MTT or medium before adding the solvent. Do not shake.
4. Leave plate covered in the dark at 18 - 24°C for at least 2 hours to overnight. Samples may be read after 2 hours, but if the readings are low and there are crystals remaining, return the plate to the dark and incubate for a longer period. Room temperature (18 - 24°C) incubation is sufficient, but incubation at 37°C may help to shorten the solubilization time.
5. Remove the plate cover and measure the absorbance of the wells, including the blanks, at 570 nm with a reference wavelength of 650 nm. If a 570 nm filter is not available, absorbance may be read with any filter in the wavelength range of 550 - 600 nm. The blanks should give values of 0 ± 0.1 O.D. units.

NOTE: You may solubilize the converted dye with 2ml acidic isopropanol (0.04 M HCl in absolute isopropanol in the ratio of 1:100) especially when MTT color interferes with your treatment. Pipette up and down several times to make sure the converted dye dissolves completely. Incubate 37°C for 30min, gently mix, and incubate 37°C for 30min.

Data collection, calculation, and interpretation

1. Determine the average values from triplicate readings and subtract the average value for the blank. Plot absorbance on the y-axis versus treatment on the x-axis. The number of cells to use in your assay should lie within the linear portion of the plot and yield an absorbance of 0.75 - 1.25.

NOTE: The cell culture conditions can affect the results, and so it must be taken into consideration when analyzing the data. The age of the culture, number of passages, and the details of the growth medium can all be important factors. Nat ural variation in the requirements and growth rates of different cell lines make it difficult to provide precise guidelines for preparing your cells.

NOTE: The plot of data obtained from the first procedure (Determination of optimal Cell Count) should provide a curve that has a linear portion. Selection of a cell number that falls within the linear portion of the curve (i.e. providing values between the range of 0.75 and 1.25) allows for the measurement of both stimulation and inhibition of cell proliferation.

NOTE: If the absorbance values of the experimental samples are higher than the negative control cells, this indicates an increase in cell proliferation. Alternatively, if the absorbance rates of the experimental samples are lower than the nega tive controls, this indicates a reduction in the rate of cell proliferation or a reduction in overall cell viability.

NOTE: In some rare instances, an increase in cell proliferation may be offset by cell death. Therefore, evidence of cell death may be inferred from morphological changes.

2 Absorbance values that are lower than the control cells indicate a reduction in the rate of cell proliferation. Conversely a higher absorbance rate indicates an increase in cell proliferation. Rarely, an increase in proliferation may be offset by cell death;

Storage & Handling

- Upon receipt, the kit should be stored at 4°C protected from light. Stored properly, the kit components should remain stable for 12 months.

References

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