Cell Viability Assays

Introduction

Tetrazolium compounds used to detect viable cells fall into two basic categories:

1.Positively charged compounds (MTT) that readily penetrate viable cells:

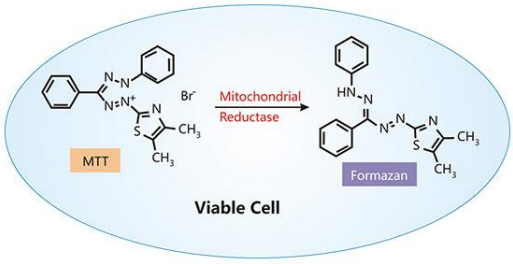
Viable cells with active metabolism are able to convert MTT into a purple-colored formazan product. Thus, color formation can be a useful marker of viable cells. However, the incubation time for this method is long (usually 4 hours). Also, the formazan product is insoluble, so a solubilizing reagent must be added prior to recording absorbance readings.

2.Negatively charged compounds (MTS, XTT, WST-8) that do not penetrate cells:

Negatively charged compounds must be combined with intermediate electron coupling reagents, which can enter cells, be reduced and then exit the cell to convert tetrazolium to the soluble formazan product. The incubation time for this method is 1–4 hours. There is no need to add a solubilizing reagent since the resulting formazan is soluble, making it more convenient.

Protocol

MTT assay protocol



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 (Figure 1). The viable cells contain NAD(P)H-dependent oxidoreductase enzymes which reduce the MTT to formazan. The insoluble formazan crystals are dissolved using a solubilization solution and the resulting-colored solution is quantified by measuring absorbance at 500-600 nanometers using a multi-well spectrophotometer. The darker the solution, the greater the number of viable, metabolically active cells.

MTT reagent preparation

Prepare MTT Solution

1. Dissolve MTT in Dulbecco's Phosphate Buffered Saline, pH=7.4 (DPBS) to 5 mg/ml.
2. Filter-sterilize the MTT solution through a 0.2 µM filter into a sterile, light protected container.
3. Store the MTT solution, protected from light, at 4°C for frequent use or at -20°C for long term storage.

Prepare MTT solvent

4 mM HCl, 0.1% NP40 in isopropanol

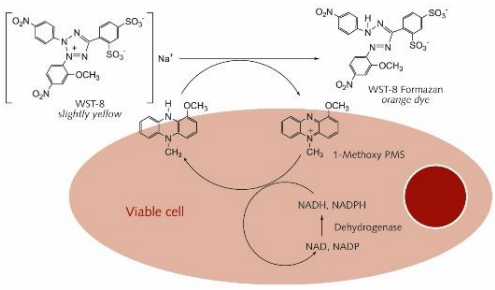
Solubilization solution

1. Choose appropriate solvent resistant container and work in a ventilated fume hood.
2. Prepare 40% (vol/vol) dimethylformamide (DMF) in 2% (vol/vol) glacial acetic acid.
3. Add 16% (wt/vol) sodium dodecyl sulfate (SDS) and dissolve.
4. Adjust to pH = 4.7 Store at room temperature to avoid precipitation of SDS. If a precipitate forms, warm to 37°C and mix to solubilize SDS.

MTT assay protocol

1. Discard media from cell cultures. For adherent cells, carefully aspirate the media. For suspension cells, spin the 96 well plate at 1,000 xg, 4°C for 5 minutes in a microplate-compatible centrifuge and carefully aspirate the media. An alternative method is to add an equal volume of MTT solution to the existing media in the culture. Ensure that the same volume of existing media is present for each sample.
2. Add 50 µL of serum-free media and 50 µL of MTT solution into each well.
3. Incubate the plate at 37°C for 3 hours.
4. After incubation, add 150 µL of MTT solvent into each well.
5. Wrap plate in foil and shake on an orbital shaker for 15 minutes. Occasionally, pipetting of the liquid may be required to fully dissolve the MTT formazan.
6. Read absorbance at OD=590 nm. Read plate within 1 hour.

WST-8 protocol



WST-8 is a second-generation tetrazolium salt and used as a chromogenic indicator for cell viability. The reduction of slightly yellow WST-8 by viable cells produces an orange-colored formazan product, which is directly proportional to the number of viable cells in the range of 200–25,000 cells/well for many cell lines including nonadherent cells. WST-8 is found to be more sensitive for cell viability measurements than those of other tetrazolium salts including MTT, MTS, XTT, and WST-1. Furthermore, WST-8 produces water-soluble formazan upon cellular reduction, which does not require an additional step to dissolve the formazan, providing an additional advantage to the method.

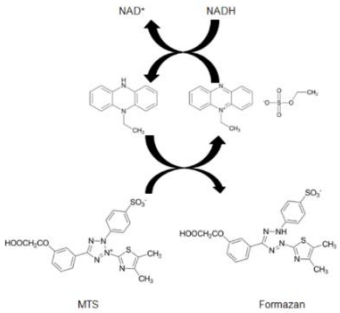
Reagent preparation

WST-8 reagent solution is prepared as an aqueous solution containing 5 mM WST-8, 0.2 mM 1-methoxy PMS, and 150 mM NaCl.

Protocol

- Cell suspensions seeded to 96-well plates (100 µl/well) with or without the test compounds are incubated at 37°C in a humidified incubator with 5% CO_2 for required exposure time.
- 10 µL of WST-8 reagent solution is added to each well and the plate is incubated at 37°C for 2 hr. After incubation, the absorbance is measured at 450 nm with a multiplate reader.

MTS protocol



The MTS assay is designed to evaluate the metabolic activity and viability of cells. The assay involves the conversion of the tetrazolium salt MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) to a purple formazan in the presence of phenazine methosulfate. The enzymes responsible are NADPH-dependent dehydrogenases, which are active in viable cells. The absorbance of the resulting formazan solution, which is proportional to the number of cells, can be quantified using a spectrophotometer. The absorbance can be measured at 490–500 nm. In addition to cell viability, MTS assays can be used to measure cell proliferation and evaluate cytotoxicity.

Reagent preparation

MTS solution (containing PES)

1. Dissolve MTS powder in DPBS to 2 mg/ml to produce a clear golden-yellow solution.
2. Dissolve PES powder in MTS solution to 0.21 mg/ml.
3. Adjust to pH 6.0 to 6.5 using 1N HCl.
4. Filter-sterilize through a 0.2 µm filter into a sterile, light protected container.
5. Store the MTS solution containing PES protected from light at 4°C for frequent use or at -20°C for long term storage.

MTS assay protocol

1. Prepare cells and test compounds in 96-well plates containing a final volume of 100 µl/well. An optional set of wells can be prepared with medium only for background subtraction.
2. Incubate for desired period of exposure.
3. Add 20 µl MTS solution containing PES to each well (final concentration of MTS will be 0.33 mg/ml).
4. Incubate 1 to 4 hours at 37°C.
5. Record absorbance at 490 nm.