CMDC Labs

CELLULAR
PROLIFERATION ASSAYS

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Commonly used methods for assessing cellular viability, proliferation, and cytotoxicity are used to evaluate how well cells in culture are responding to diverse stimuli. The right assay method should be chosen based on the number and type of cells being employed as well as the expected results. The number of cells over time, the frequency of cellular divisions, metabolic activity, or DNA synthesis can all be tracked using assays for cell proliferation. Trypan blue or Calcein-AM are two viability dyes that can be used in cell counting to determine the percentage of viable cells as well as the rate of cell growth.

DNA SYNTHESIS PROLIFERATION ASSAYS

BRDU CELL PROLIFERATION ASSAY

By tracking the incorporation of the radioisotope [3H]-thymidine into the cellular DNA and then performing autoradiography, cell proliferation can be investigated. Thymidine may also be substituted by 5-bromo-2'-deoxy-uri-clonal antibody against BrdU and a secondary antibody that has been enzyme- or fluoro- chrome conjugated, it is simple to identify cells that have integrated BrdU into their DNA.

EDU PROLIFERATION ASSAYS

An effective way to detect DNA replication using fluorescence is to use Baseclick EdU proliferation assays. Live cells are given the modified nucleoside EdU, which is then integrated into DNA that is replicating. Fluorescent probes may be attached to the EdU quickly thanks to Cu-induced click chemistry. This makes it possible to quantify the proliferation of cells. The assays are offered in a number of forms for in vivo research, flow cytometry, high throughput screening, and microscopic imaging. For multiplexing with other fluorescent probes, four alternative fluorescent probes with peak excitations of 488, 555, 594, and 647 are used.

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METABOLIC PROLIFERATION ASSAYS

The analysis of proliferation, viability, and cytotoxicity is suitable for assays that assess metabolic activity. Only cells with active metabolism can reduce tetrazolium salts like MTT, XTT, and WST-1 to colorful formazan compounds or bio-reduce resazurin. Cells that are actively growing have higher metabolic activity than cells that have been exposed to toxins.

MTT CELL PROLIFERATION ASSAYS

When made in media or salt solutions devoid of phenol red, MTT (3-[4,5 dimethylthiazol-2-yl]-2,5- diphenyltetrazolium bromide; thiazolyl blue) is a water-soluble tetrazolium salt that yields a yellowish solution. Insoluble purple formazan is created when dehydrogenase enzymes from live cells cleave the tetrazolium ring from dissolved MTT. Isopropanol or other solvents can be used to solubilize the insoluble formazan in water, and the dissolved material is then quantified spectrophotometrically using absorbance as a function of converted dye concentration. See Figure 1 for the chemical formulas.

XTT CELL PROLIFERATION ASSAYS

Unlike MTT, XTT's cleavage product is soluble in water; as a result, solubilization is not necessary.

A sophisticated cellular process converts the tetrazolium salt XTT to formazan.

Only live cells do this bio reduction, which is linked to the glycolytic synthesis of NAD(P)H.

The number of metabolically active cells in the culture is directly correlated with the amount of formazan dye produced.

WST-1 CELL PROLIFERATION ASSAYS

A complicated cellular mechanism that predominantly takes place at the cell surface breaks down the stable tetrazolium salt WST-1 into a soluble formazan.

The generation of NAD(P)H by glycolysis in functional cells is crucial for this bio-reduction.

The number of metabolically active cells in the culture is directly correlated with the amount of formazan dye produced.

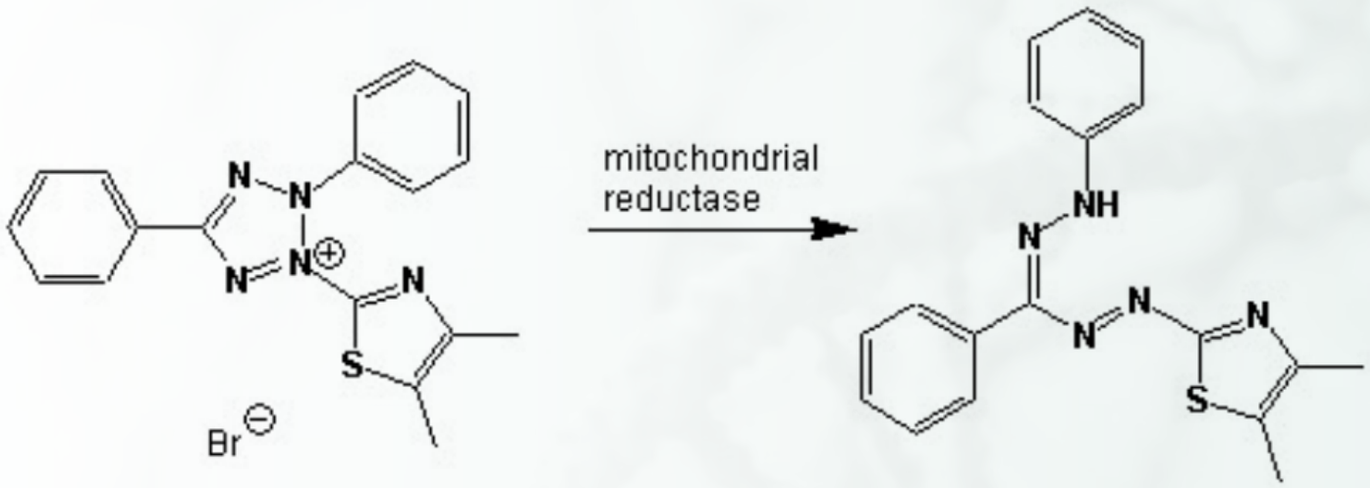

Figure 1.
Based on metabolic activity, the MTT assay is a colorimetric technique for evaluating cell growth.

Cellular ox- idoreductase enzymes that areNAD(P)H-dependent show how many live cells are present. These enzymes may convert the yellow tetrazolium dye MTT 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide into the insoluble purple formazan.

LUMINESCENT CELL VIABILITY ASSAYS

The quantity of ATP available can be used to estimate the number of live cells because ATP is a marker for metabolically active cells. For estimating ATP levels in cell cultures, the ATP Cell Viability Luciferase Test provides a very sensitive homogeneous assay. This test uses the oxidation of D-Luciferin by firefly luciferase and the subsequent creation of light to determine the quantity of ATP present in cell cultures. A single addition of the ATP detection cocktail to cells grown in a medium supplemented with serum is required for the sensitive assay process. There is no need to remove the media, wash the cells, or pipette more than once. The test has a sensitivity of 0.01 picomoles for ATP or a single cell. Within six orders of magnitude, the signal is linear. The assay has a wide range of applications, from counting the number of viable cells to measuring cell proliferation and cytotoxicity, since it links the amount of ATP to the quantity of live cells.

FLUORESCENT DYE PROLIFERATION ASSAYS

CFSE LABELING

A common choice for counting the number of divisions a population of cells undergoes is 5(6)-Carboxyfluorescein diacetate N-succinimidyl ester (CFSE). Intracellular esterases break down CFSE as soon as it enters the cell, releasing the fluorescent molecule, and the succinimidyl ester group interacts covalently with primary amines on intracellular proteins.
The fluorescence intensity of each daughter cell is halved during cell division, making it easy to count the number of divisions using flow cytometry. CFSE has been frequently used to assesslymphocyte proliferation, especially for that of T cells.

LIVE/DEAD CELL DOUBLE STAINING

Live/Dead Cell Double Staining can be used to simultaneously detect both living and dead cells using fluorescence. Calcein-AM is a dye that is extremely lipophilic and permeable to cell membranes.
Although Calcein-AM itself is not luminous, Calcein-AM is converted into calcein by the enzyme esterase in a living cell, which produces a bright green fluorescence (Ex 490 nm, Em 515 nm). Therefore, Calcein-AM only stains healthy cells. In contrast, a healthy cell membrane blocks the nuclei-staining dye Propidium Iodine. Through disorganized regions of the dead cell membrane, it enters the nucleus where it intercalates with the DNA double helix to emit red fluorescence (Ex 535 nm, Em 617 nm).

A single-excitation fluorescence microscope can be used to simultaneously monitor live and dead cells because both calcein and PI-DNA can be activated by 490 nm light.

3D CELL CULTURE
LIVE/DEAD/TOTAL CELL TRIPLE STAINING

The Cell Viability Imaging Test is a three-color assay for simultaneous fluorescence labeling of viable cells (Calcein-AM), dead cells (Propidium Iodide/PI), and total cells (Hoechst 33342). It can be utilized with 2D and 3D cell cultures.

- Calcein-AM fluoresces green on binding calcium, relying on esterase activity present only in metabolically-active viable cells.

- Propidium Iodide (PI) is a nuclear dye that is excluded by the membrane of live cells, but passes through the damaged membrane of dead cells, intercalating with the DNA to emit a strong red fluorescence only in dead cells.

- Hoechst 33342 is a DNA staining dye that exhibits low cytotoxicity. It fluoresces blue and is used as an indicator of total cell count.

TRYPAN BLUE CELL COUNTING

One of the stains suggested for use in dye exclusion techniques for viable cell counting is trypan blue. This technique is based on the idea that while dead (non-viable) cells absorb the blue dye, live (viable) ones do not. The ratio of total living cells to total cells can be used to calculate cell viability (live and dead). Additionally, staining makes it easier to see a cell's general morphology

Remarkably, serum proteins have a stronger affinity for Trypan Blue than do cellular proteins. Prior to counting, cells should be pelleted and resuspended in protein-free media or salt solution if the background is excessively dark because serum is present in the matrix.

Making the Unknown Known...