Cell Counting

Protocol

Bulletin 6234

Cell Viability and Cytotoxicity

Quantifying cell growth in terms of proliferation and viability is an essential tool for optimizing cell cultures, assessing the efficiency of molecules in screening, or evaluating the cytotoxicity of compounds for treating cancer. Cell viability and cytotoxicity can be assessed by measuring the amount of live and dead cells in a total cell sample. This can involve either counting the number of live cells in a sample or by measuring an indicator for healthy cells in a population. There are several methods available for these measurements, including fluorescence based detection, non-fluorescence based detection and chemiluminescence based detection.

Fluorescence based detection (Millard P et al. 1997)

One way to distinguish live cells from dead cells is by the presence or absence of esterases (Burghardt R, et al. 1994). Live cells contain esterases and are thus able to convert non-fluorescent esterase substrates into intensely fluorescent molecules and their intact intracellular membrane retains the cleaved fluorescent products inside the cell. Dead cells, on the other hand, are deficient in esterase activity and their compromised membranes lead to substrate leaks from cells. Cell-permeable esterase substrates, including calcein AM and fluorescein diacetate, can be used to not only measure esterase activity but also cell membrane integrity within a cell sample (Jones KH and Senft JA 1985).

An alternate method of quantification utilizes non-permeable nucleic acid stains. These stains are only fluorescent when bound to nucleic acids of eukaryotic or prokaryotic cells and are commonly used in combination with esterase substrates.

A third method detects live and dead cells based solely on cell permeability. Typical examples include impermeable dead cell indicators like ethidium homodimer and propidium iodide, or cell permeable calcein AM staining.

Non-fluorescence based detection

Colorimetric assays are based on a color change caused by the structural differences or metabolic impairment between live and dead cells. Assessment is based on retention of certain dyes or exclusion of others. For example, Trypan blue is useful in dye-exclusion because the cell membrane of live cells are intact and prevent the Trypan blue dye from entering, therefore live cells remain bright. Dye enters dead cells through their compromised cell membranes and colors them blue. By contrast, neutral red is a vital stain that concentrates in lysosomes of living cells, live cells become red and dead cells remain bright.

Assays of metabolic activity are based on reduction of tetrazolium salts by mitochondrial enzymes into a colored formazan. Mitochondrial enzymes are inactivated shortly after cell death making this a reliable method for detecting live cells. Commonly used tetrazolium salts include MTT, XTT, and WST-1 (Tominaga, H 1999).

Chemiluminescence based detection

The presence of ATP (adenosine 5'-triphosphate) is an indicator of live cells and can be used to quantify live cells in a sample, monitor cell proliferation or cytotoxicity, and detect bacterial contamination. Although different methods of quantifying ATP have been developed, chemiluminescent detection with luciferase is the most sensitive assay. Luciferins are small molecules substrates for specific luciferase enzymes and are naturally present in some organisms, including the firefly (Ford SR, et al.1996).

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Abbreviations

ATP	adenosine 5'-triphosphate
WST-1	4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfonate
XTT	2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxanilide
MTT	3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide

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