Product Name

Name: Trypan Blue Solution, 5 mg/mL in Saline

Cat. No.: C3730-0100

Size: 100 mL

Product Description

Trypan Blue Solution, 5 mg/mL in Saline is an acid diazo dye of the benzopurpurine series derived from toluidine. These dyes, derivatives of toluene, are most commonly utilized as a vital stain to distinguish viable from non-viable cells. The reactivity of trypan blue is based on the fact that the chromophore is negatively charged and hence does not interact with the cell unless the cytoplasmic membrane integrity is challenged. Therefore, viable cells selectively exclude the dye, and hence the uncompromised and intact cell membrane does not absorb the color while non-viable or dead cells permit the dye to traverse or permeate the membrane and appear distinctively blue under a microscope. Since live cells are excluded from staining, this particular staining method is also known as the Dye Exclusion Assay (DEA). DEA is a relatively simple, yet reliable method for the quantification or estimate of cell viability in a suspension where non-viable cells will be stained and healthy cells can be determined by direct count with a hemacytometer. Cells should be prepared in a suspension of single cells in buffered saline before counting. As trypan blue has a higher affinity for serum protein than for cellular proteins, suspending cells in a serum-containing medium will generate a darker background. When interpreting results, caution should be used as the dye uptake is pH- and concentration-dependent, and therefore, under certain circumstances, results may be misleading if protocols are not followed judiciously.

Cell Viability (CV)

Determining cell viability and proliferation is part and parcel of quantification of cellular growth and is an essential tool in any cell-based research. In any given cell culture experiment, a major decision is whether to test for viability or for cell proliferation. Such parameters are measured by assaying the co-called "vital functions" that are characteristic of healthy cells in a sample. This dye exclusion assay is based upon the concept that intact, viable cells are impermeable to vital dyes, whereas dead cells are permeable to the dyes. Trypan blue is the most commonly used dye for this purpose. Cell viability may be accomplished by directly counting the number of healthy cells. Whether cells are in an active or quiescent phase can be determined by many other methods; however, an increase in cell viability is indicative of cell growth, while a decrease in viability is indicative of two basic and fundamental problems:

- Sub-optimal culture conditions and/or
- Toxic shock

Indirect measurements of cell viability are based on cellular metabolic activity. The most commonly used parameter is glucose utilization although other parameters such as lactic, pyruvic acid or CO_2 production, O_2 utilization, or mitochondrial enzymatic activity may be used. When cells are growing logarithmically, there is a close correlation between cell numbers and nutrient utilization.

Today, cell viability assays are based on either uncompromised cytoplasmic membrane integrity or cell

metabolic activity. In sum, not only metabolic activity is measured in cell populations via incubation with a tetrazolium salt (e.g., XTT) which is cleaved into a colored formazan product by metabolically active cells, but also ATP status may be analyzed and is indicative of the energy capacity of cells and hence their viability.

Cell Count

Although the cell count is an absolute and direct measure of proliferation, DNA content, or metabolic activity measurements are all correlates that can be altered by factors other than cell count. Metabolic activity is still a much better indicator of cellular health.

Review of the Principles of Cell Proliferation Assay (CPA)

Whereas the dye exclusion assay (DEA) is a more direct, very approximate, and superficial estimate of cell viability in a suspension, indirect measurement methods of CV should be performed when a more precise quantitation is demanded as the measurement of cellular metabolic activity is a much more precise marker of cell dynamics. CPA is based on the ability of metabolically active cells to reduce the tetrazolium salt, XTT, to orange-colored compounds of formazan. The resulting intensity of the water-soluble dye can be read at a given wavelength by spectrophotometric methods. The intensity of the dye is proportional to the number of metabolically active cells. The use of multi-well plates and an ELISA plate reader enables testing on a much larger scale while obtaining straightforward and rapid results. The test procedure includes cell culture in a 96-well plate with the addition of the XTT reagent and incubation from 2 - 24 hours. During incubation, an orange color is formed and therefore, its intensity can eventually be measured by spectrophotometric methods. In conclusion, the greater the number of metabolically active cells in the well demonstrates an elevated level of mitochondrial enzymatic activity which correlates with the concentration of the dye produced which can be quantified easily.

Some Predominant Characteristics of Trypan Blue Solution, 5 mg/mL in Saline include:

- Liquid formulation
- Vital stain for estimation of cell viability
- Dye exclusion method
- Sterile-filtered (0.1µm), cell-culture tested

Storage and Stability

The product should be kept at **15 - 30°C**.
The product is **light-sensitive** and therefore should not be left in the light.
Shelf life: 24 months from date of manufacture

Procedure

1. Aseptically withdraw a sample of the cell suspension and prepare 1:2, 1:5, 1:10, or 1:100 dilutions as required in PBS. Dilute 1:5 in 0.5% Trypan Blue solution. The optimal concentration of cells for counting is 5 – $10*10^5$ cells/mL (50-100 cells per large square of the hemocytometer counting chamber) after dilution in Trypan Blue Solution.

2. After staining with Trypan Blue, the cells should be counted within three minutes; after that interim, the non-viable cells will begin to take up the dye.
3. Using a Pasteur pipette, withdraw a small amount of the stained cell suspension and place the tip of the pipette onto the slot between a clean hemocytometer and a planar coverslip, thereby creating a three-dimensional space. The cell suspension will be siphoned under the coverslip by capillary action as the fluid is allowed to flow from the pipette under the coverslip to cover the whole grid. Next fill the opposite chamber with the second diluted sample. Do not overfill the chamber and do not disturb the coverslip after the hemocytometer has been "charged."
4. Place the hemocytometer on the stage of an inverted microscope and observe with a 10X objective. Adjust the focus until a single counting square fills the field. The etched grid marks the boundaries for the counting and delineates a specific volume within the space.

Precaution and Disclaimer

For research use only, not for clinical diagnosis, and treatment.