Product Profile

Product Name:	*Trypan Blue Solution,5mg/ml in Saline*
Product Catalog Number	*03-102-1*
Unit Size Availability:	(B)100ml
Concentration:	*1X*
Formulation:	Blue-Colored Solution
Specified Storage Conditions:	Room Temperature(15-30°C)
Stability: (Under Specified Handling & Storage)	*Please Refer to the Product Label*

Important Note! Please read the *MSDS* and *Product Profile* carefully in their entirety *before* using this material for possible safety precautions and potential hazards.

Product Description:

Trypan Blue Solution,5mg/ml in Saline is an acid diazo dye of the benzopurpurine series derived from toluidine, any one of several isomeric bases, $C_{14}H_{16}N_2$. These bases, 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 chromopore 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 the non-viable or dead cells permit the dye to traverse or permeate the membrane and appear distinctively blue or orthochromatic in color 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 by hemacytometry. Cells should be in suspension as single cells in buffered saline before counting. As Trypan Blue has a higher affinity for serum protein than for cellular proteins, suspending such cells in medium containing serum 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 parameters 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 certain dyes, whereas dead cells are subject to its vagaries. Typan blue is the most commonly used dye for this purpose. Cell viability may be accomplished by directly counting the number of healthy cells. Whether the cells are in an active or quiescent phase is difficult to determine, however an increase in cell viability is indicative of cell growth, while a viability decrease 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 enzymatic product expression may be used. When cells are growing logarithmically, there is a close correlation between cell numbers and nutrient utilization. Still when using indirect measurements, it is incumbent to distinguish between different growth phase maintenance demands when component utilization levels increase versus interpreting actual growth with a concomitant or parallel increase in component 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) that is cleaved into a colored formazan product by metabolically active cells, but also, ATP cell status may be analyzed and is indicative of 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 a much better indicator of cell health.

Review of Cell Proliferation Assay (CPA) Principles

Whereas the Dye Exclusion Assay (DEA) is a more direct and 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 cell metabolic activity is a much more precise marker of cell dynamism. CPA is based on the ability of metabolically-active cells to reduce the tetrazolium salt, XTT (i.e. as aforementioned), to orange-colored compounds of formazan. The resulting water-soluble dye's intensity 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 Reader enables testing on a much larger scale while obtaining straightforward and rapid results. The test procedure includes cell cultivation in a 96-well plate with the addition of the XTT reagent and by incubation from 2-24 hours. During the incubation process, an orange color is formed and therefore, its intensity can eventually be measured by Spectrophotometric methods. In conclusion, the greater the number of active cells in the well demontrates the elevated level of mitochondrial enzymatic activity relative to the concentration of the dye produced which then can be quantified and measured.

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

- Liquid Formulation
- Vital Stain for Estimation of Cell Viability
- Dye Exclusion Method
- More Uniform & Consistent Media Performance
- Sterile-Filtered(0.1µ),Cell-Culture-Tested

Storage, Handling, Stability Precautions and Disclaimer:

For *in vitro* diagnostic use only.

Trypan Blue Solution,5mg/ml in Saline is stable when stored under defined conditions at 15-30°C. The product is light-sensitive and therefore should not be left in the light. When stored in the dark under ideal conditions, the product is stable until the expiry date.

As with any other liquid media formulations, *deterioration of liquid media* may be recognized by any of the following characteristics, among others including: (a). Color Change, (b). Presence of clumping/flocculent debris/ granulation/ particulates\ precipitates or sediments (c). Insolubility, (d). And/or decrease in expected performance parameters. Any material described above should not be used and therefore discarded.

Instructions/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. The optimal concentration of cells for counting is 5-10X10^5 cells/ml (50-100 cells per large square of the hemocytometer counting chamber) after dilution in the Trypan Blue Solution.

2) After staining with Trypan Blue, the cells should be counted within three (3) 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 of a clean hemocytometer with a planar coverslip, thereby creating a three-dimensional space. The cell suspension will be transferred under the coverslip by capillary action as the fluid is allowed to flow from the capillary under the coverslip to cover the area of the 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 using the 10X objective. Adjust focus until a single counting square fills the field. The etched grid marking the boundaries for the counting procedure delineates a specific volume within the space.

Quality Control

Test	Specification
Sterility:	Sterile

Auxiliary Products

Product Name	Catalog Number	Storage Temperature
DMEM, with 1g/l D-Glucose(Low Glucose) ,with Sodium Pyruvate (110mg/l),without L-Glutamine	01-050-1	2-8°C
DMEM Low Glucose 5X, without L-Glutamine, without Sodium Bicarbonate	01-050-4	15-30°C
DMEM with D- Glucose 4500mg/L,without Sodium Pyruvate, without L-Glutamine, without Phenol Red	01-053-1	2-8°C
DMEM with D- Glucose 4500mg/L,without Sodium Pyruvate, without L-Glutamine, without L-Methionine	01-054-1	2-8°C
DMEM with D- Glucose 4500mg/L,without Sodium Pyruvate, without L-Glutamine	01-055-1	2-8°C
DMEM High Glucose 2X, without L-Glutamine, with Sodium Bicarbonate	01-055-9	2-8°C
Quick Stain	01-939-1	2-8°C
Dulbecco's Phosphate Buffered Saline(DPBS)	02-020-1	2-8°C
Dulbecco's Phosphate Buffered Saline(DPBS) ,without Calcium and Magnesium	02-023-1	15-30°C
Dulbecco's Phosphate Buffered Saline(DPBS), 10X Conc.,without Calcium and Magnesium	02-023-5	15-30°C
L-Glutamine Solution 29.2mg/ml in Saline	03-020-1	-20°C
L-Alanyl-L-Glutamine Solution(A Dipeptide Substitute)	03-022-1	-20°C
Penicillin-Streptomycin Solution	03-031-1	-20°C
Sodium Pyruvate Solution	03-042-1	-20°C
Water, Cell Culture Grade	03-055-1	15-30°C
Phenol Red Solution,5mg/ml in DPBS	03-100-1	15-30°C
Fetal Bovine Serum	04-001-1	-20°C
Fetal Bovine Serum(Qualified for Human Embryonic Stem Cells)	04-002-1	-20°C
Adult Bovine Serum	04-003-1	-20°C
DMEM with D- Glucose 1000mg/L, with Sodium Pyruvate 110mg/L, with Hepes(20mM)	06-1050-18-1	2-8°C
Cell Proliferation Kit(XTT Based)	20-300-1000	-20°C
Note: For a list of other Antibiotics, Serum, other Supplements or Reagents, please refer to our Product Catalog/Product Profiles/ Product Guides and Internet Site.		

References:

1) Biological Industries (BI) Specifications
2) Darling, D.C. and Morgan S.J. Animal Cells: Culture and Media, John Wiley & Sons, New York, 1994
3) O'Neil, Maryadele *et. al*., The Merck Index, 14th Edition, Whitehouse Station, New Jersey,2006
4) Lackie, J. M. The Dictionary of Cell & Molecular Biology, Academic Press: London, 2007