Trypan Blue (0.4% solution)
For research use only

Catalogue number: BI-1803

Product Description

Trypan blue is an azo dye, derived from toluidine. It is also known as diamine blue and Niagara blue. It is widely used as a vital stain to distinguish viable from non-viable cells. It selectively stains the dead tissues or cells blue in color. Live cells or tissues, with intact cell membrane, are not colored, and as a result, trypan blue is not absorbed by viable cells. However, the dye can pass through the cell membrane of dead cells and makes them to appear blue (and countable) in color under the microscope. Since live cells are excluded from the staining, this method is also described as a dye exclusion method. Trypan blue is most commonly used in microscopy for cell counting. This product is 0.4% solution of Trypan blue in Dulbecco's Phosphate Buffered Saline. The products is filtered through 0.2µm filter to remove debris.

Specification

- **Appearance:** Dark blue liquid
- **pH:** 7.00 -7.80
- **Sterile**
- **Shelf life:** 12 months
- **Storage:** 20-25ºC.

How to Use

1. Prepare a suspension of approximately 1 × 10^6 cells/mL. Ensure that the sample is thoroughly mixed.
   NOTE: Adherent cells must first be treated with trypsin to create a cell suspension. A concentration of 1X 10^6 cells/mL will result in an average of 50 cells per corner grid of the hemocytometer, which is a reasonable cell count for accuracy and precision. A concentration of 1 × 10^5 cells/mL will be too low to produce an accurate count. This sample should be concentrated by centrifugation before counting. A concentration of 1X 10^7 cells/mL will be too high to count accurately and should be diluted before counting.
2. Make a 1:1 mixture of the cell suspension and the 0.4% trypan blue solution. The sample can be as small as 10 mL to several mL in volume. Gently mix and let stand for 5 min at room temperature.
   NOTE: Do not leave cells in trypan blue for more than 15 min, in order to prevent cell death due to trypan blue toxicity.
   NOTE: Trypan Blue is a potential mutagen. Handle the dye with care and dispose off the waste safely as per applicable local regulations.
3. Prior to use, wash the hemocytometer with 70% (v/v) ethanol and allow to dry.
4. Wash a coverslip with 70% (v/v) ethanol, allow to dry, and place on top of the hemocytometer counting chamber.
5. Apply 15 mL of cell suspension to the edge of the chamber between the cover slip and the V-shaped groove in the chamber. Allow the cell suspension to be drawn into the chamber by capillary action.

6. Let sit for 1–2 min and then count.

   NOTE: If greater than 10% of the cells appeared clustered, repeat the entire procedure to make sure that the cells are dispersed by vigorous pipetting in the original cell suspension (as the Trypan Blue cell suspension mixture). If less than 200 or greater than 500 cells (i.e., 20-50 cells/square) are observed in the 10 squares, repeat the procedure adjusting to an appropriate dilution factor.

7. Calculate the percentage of viable cells as follows:

$$\text{viable cells}(\%) = \frac{\text{total number of viable cells per mL of aliquot}}{\text{total number of cells per mL of aliquot}} \times 100$$

To obtain the total number of viable cells per mL of aliquot, multiply the total number of viable cells by 2 (the dilution factor for trypan blue). To obtain the total number of cells per mL of aliquot, add up the total number of viable and nonviable cells and multiply by 2.

References

1. Olivares-Reyes, Jesús Alberto, et al. "Oxidative stress induced by P2X7 receptor stimulation in murine macrophages is mediated by c-Src/Pyk2 and ERK1/2." Biochimica et Biophysica Acta 2013 (1830): 4650-4659.
2. Ha-Duong, Nguyêt-Thanh, Miryana Hémadi, and Jean-Michel El Hage Chahine. "Transferrin receptor-1 iron-acquisition pathway—Synthesis, kinetics, thermodynamics and rapid cellular internalization of a holotransferrin–maghemite nanoparticle construct." Biochimica et Biophysica Acta 2013 (1830): 4254-4264.
3. Strober, W. 1997. Current Protocols in Immunology. A.3B.1-A.3B.2.
4. Kristine S. Louis and Andre C. Siegel, Cell Viability Analysis Using Trypan Blue: Manual and Automated Methods.