Trypan Blue Solution

Instruction Manual

Catalog Number	PK-CA902-1209
Description	Trypan Blue is the stain most commonly used for dead cell staining and to distinguish viable from nonviable cells. It is a water-soluble dye used for the dye exclusion test for cell viability and distinguish between viable and nonviable cells by seeping into nonviable cells with damaged plasma membranes causing them to appear blue. Viable cells exclude the dye and are not stained by Trypan Blue while nonviable cells absorb the dye and appear blue. Therefore, dead Trypan Blue-stained cells are easily recognized by microscopy and can be counted using a hematocytometer. Erythrosin B, negrosine, eosin Y, AO and EB are also utilized for this purpose. Though it is hard to detect cells in early to middle stages of apoptosis, Trypan Blue staining is a very simple and widely used method to visualize dead cells.Do not leave on cells for long periods of time; viable cells may also begin to take up the dye.
Quantity	10 ml (0.5%)
Sequence / Molecular Weight / Molecular Formula	NA
Purity	NA
Appearance / Formulation / Solubility	Liquid. Supplied as a 0.5% (w/v; 5 mg/ml) solution in normal saline. Filter sterilized and packed under sterile conditions.
Storage & Stability	Store at 4°C.
Applications	Note: Cells should be in suspension as single cells in buffered saline before counting. Trypan Blue has a higher affinity for serum protein than for cellular proteins, so suspending cells in medium containing serum will generate a dark background. 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 5-10 x 10^5 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.
References	NA
Caution	NA

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