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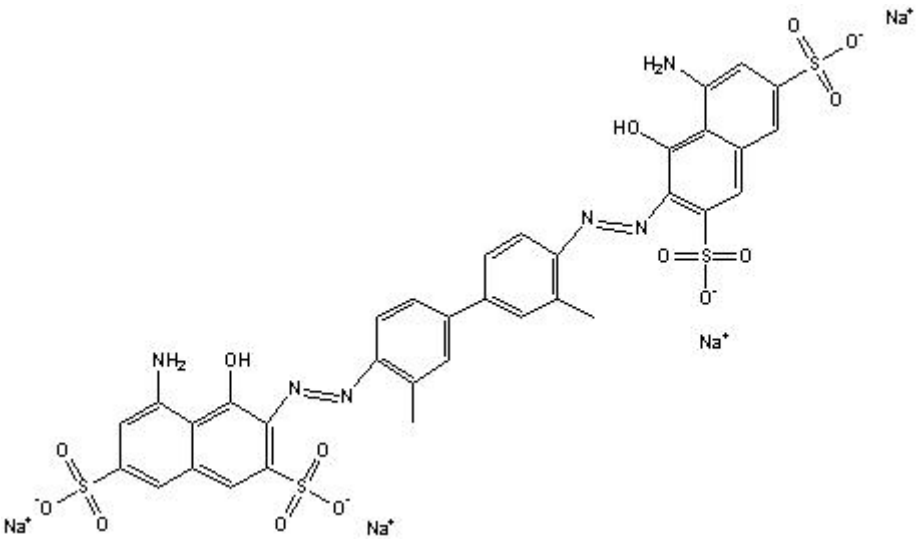
TECHNICAL INFORMATION

Catalog Number: 195532
Trypan Blue

Structure:



Molecular Formula: $C_{34}H_{24}N_6Na_4O_{14}S_4$

Molecular Weight: 960.82

CAS # : 72-57-1

Synonyms: Direct Blue 14; Niagara Blue 3B; Chlorazol blue 3B; Benzo blue 3B; Dianil blue H3G; Congo blue 3B; Naphthamine blue 3BX; Benzamine blue 3B; Azidine blue 3B; Diamine Blue 3B

C.I. 23850

Physical Description: Dark Blue powder (195532 or 194600); Dark Blue Liquid (16910)

Solubility: Soluble in ethylene glycol monomethyl ether (methyl cellosolve; EGME) (20 mg/ml); Slightly soluble in water or ethanol (0.6 mg/ml); almost insoluble in alcohol

Formulation (for #16910 - Trypan Blue (0.4%) in PBS):

Components		***Mol. Wt.***	***Mol. (mM)***
Inorganic Salts	***mg/L***		
Potassium Phosphate Dibasic		174.18	3.44
Sodium Chloride [NaCl]	600.00000	58.44	138.60
Other	8100.00000		

Trypan Blue	4000.00000	960.8	4.16

Description: A blue acid dye with a strong affinity for cellulose containing substrates such as cotton; less affinity for proteinaceous materials. Used as a vital dye which is especially important because it is taken up by the reticuloendothelial system.[3]

Uses: Clark[2] describes assays for the study of teratogenic action of trypan blue on embryonic tissues using Davis and Sauter's fluorescence method and for the staining of collagen, including very fine fibrils, muscle and cornified epithelium using the Van Gieson method.

Trypan blue is also recommended for use in dye exclusion procedures for viable cell counting. Non-viable cells will up-take trypan blue at a faster rate than viable cells.

Typical Procedure for Viable Cell Counting:

Note: Trypan blue has a greater affinity for serum proteins than for cellular protein. If the background is too dark, cells should be pelleted and resuspended in protein-free medium or salt solution prior to counting.

Reagents:

A. 0.4% (w/v) Trypan Blue Solution in saline or PBS or similar buffer.
B. A Balanced Salt Solution such as Hank's Balanced Salts

Procedure:

– Prepare a cell suspension in a balanced salt solution.
– Transfer 0.5 ml of a 0.4% Trypan Blue Solution to a test tube. Add 0.3 ml of the balanced salt solution and 0.2 ml of the cell suspension (dilution factor = 5) and mix thoroughly. Allow to stand for 5 to 15 minutes. **Note:** Do not let this stand for too long because viable cells will also start to up-take trypan blue.
– With the cover-slip in place, use a Pasteur pipette or other suitable device to transfer a small amount of trypan blue-cell suspension mixture to both chambers of a hemocytometer (or other suitable cell counting device). Carefully touch the edge of the cover-slip with the pipette tip and allow each chamber to fill by capillary action. Do not overfill or underfill the chambers.
– Starting with chamber 1 of the hemocytometer, count all the cells in the 1 mm center square and four 1 mm corner squares. Non-viable cells will stain blue. Keep a separate count of viable and non-viable cells. **Note:** Count cells on top and left touching middle line of the perimeter of each square. Do not count cells touching the middle line at bottom and right sides.
– Repeat this procedure for chamber 2. **Note:** If greater than 10% of the cells appear clustered, repeat entire procedure making sure the cells are dispersed by vigorous pipetting in the original cell suspension as well 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.
– Withdraw a second sample and repeat count procedure to ensure accuracy.

Calculation:

Cell Counts: Each square of the hemocytometer, with cover-slip in place, represents a total volume of 0.1 mm^3 or 10^{-4} cm^3. The subsequent cell concentration per ml (and the total number of cells) will be determined using the following calculations:

Cells per ml = (the average count per square) x (dilution factor) x (10^4) [count 10 squares]

Total Cells = (cells per ml) x (the original volume of fluid from which cell sample was removed).

$$\text{Cell Viability (\%)} = \frac{\text{Total Viable Cells (Unstained)}}{\text{Total Cells (Stained and Unstained)}} \times 100$$

Availability:

Catalog Number	Description	Size
194600	Trypan Blue, Cell Culture Reagent	5 g 25 g 100 g
195532	Trypan Blue	5 g 25 g 100 g
1691049	Trypan Blue Solution, 0.4% (w/v) in PBS	100 ml

References:

– Udenfriend, S., *Fluorescence Assay in Biology and Medicine*, Academic Press: New York (1962).
– Clark, G., *Staining Procedures,* **4th Ed.,** Williams and Wilkins: Baltimore, pp. 85, 115 (1981).
– Lillie, R.D. (ed.), *H.J. Conn's Biological Stains***, 9th Ed.,** The Williams and Wilkins Company, p. 158 (1977)
– Curtis, F., "Methode de coloration elective du tissu conjonctif." *C.R. Soc. Biol. (Paris)*, **v. 58**:1038-1040 (1905).