Trypan Blue 0.4% Solution

(Cat. # 786-1383, 786-1384)

INTRODUCTION

Trypan Blue 0.4% Solution is commonly used as a cell stain to measure cell viability using dye exclusion method. The dye exclusion method is based on the principle that cell impermeable dyes like Trypan blue will stain only dead cells where as viable cells will not be stained.

Trypan Blue staining is routinely used when counting cells with hemocytometer during subculturing of cells. It can also be used anytime when cell viability need to be determined accurately and quickly.

ITEM(S) SUPPLIED

Cat. #	Description	Size
786-1383	Trypan Blue 0.4% Solution	50 ml
786-1384	Trypan Blue 0.4% Solution	2 x 50 ml

STORAGE CONDITIONS

Trypan Blue 0.4% Solution is supplied at ambient temperature. Upon arrival store at ambient temperature. The product is stable for 1 year when stored as instructed.

WARNING

Trypan Blue is a potential mutagen. Handle the dye with care and dispose off the waste safely as per applicable local regulations.

SPECIFICATIONS

- Solution is made in PBS, pH 7.4 ± 0.1
- Sterile filtered
- Store at ambient temperature

ADDITIONAL ITEMS REQUIRED

- Hemocytometer
- Microscope
- Cells

PROTOCOL

Trypan Blue viability Test

1. Dilute the cell suspension sample in ratio 1:1 with Trypan Blue 0.4% Solution
   NOTE: *Trypan Blue binds to serum proteins. If the background is too dark use HBSS or D-PBS for cell sample dilution.*
2. Place the coverslip on hemocytometer chambers and carefully fill them with Trypan Blue treated cells.
   NOTE: *Do not over-or under-fill the chamber.*

3. Incubate the cells with hemocytometer for 1-2 minutes at room temperature.
   NOTE: *For longer incubations place hemocytometer in humid chamber. Do not exceed incubation time of more than 30 minutes as viable cells will also pick up the stain after 30 minutes.*
4. Place the hemocytometer under microscope and count the cells in four 1 x 1 mm squares of one chamber and determine the average number of cells per square.
   NOTE: *For accurate cell count cells number of cells in 1 x1 mm square should range 20-50. If > 50 cells are observed the dilute the cell suspension further. If less than 20 cells are seen use undiluted sample.*
   NOTE: *Make sure that you have single cells suspension with <10 % clusters for accurate results.*
5. Determine cell count and cell viability

Cell Count per ml = Average count per square x dilution factor x 10^4 cell /ml

$$\textbf{Cell Viability (\%)} = \frac{\text{Total viable cells (unstained) x 100}}{\text{Total cells (unstained + stained)}}$$

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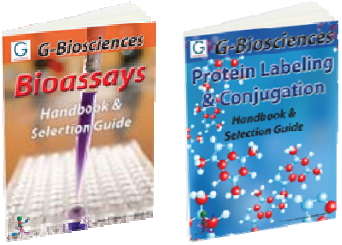

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