

Trypan Blue 0.4% Solution Troubleshooting Guide

100 mL, 5 x 100 mL

Cat No: TRY-B100, TRY-B1005

Use this guide for common counting and dye-exclusion problems with Trypan Blue 0.4% Solution. The datasheet recommends a 1:1 mixture of cell suspension and Trypan Blue, 1-2 minutes incubation at room temperature, loading into a hemocytometer, and microscopic counting. Dead cells stain blue while viable cells remain unstained.

Before You Start - Quick Checks

1. Store at room temperature.
2. Mix cell suspension 1:1 with Trypan Blue 0.4% Solution.
3. Incubate for 1-2 minutes at room temperature before counting.
4. Place the coverslip on the hemocytometer chamber before loading the mixture.
5. Count cells under a microscope and calculate cell count and viability with the appropriate formulas.
6. Handle carefully: the datasheet warns that Trypan Blue is a potential mutagen and should be disposed of according to applicable local regulations.

Problem	Most likely cause	Recommended action
Dead and viable cells are difficult to distinguish	The 1:1 dye-to-cell mixture or 1-2 minute incubation may not have been followed.	Prepare a fresh 1:1 mixture and incubate for 1-2 minutes at room temperature before microscopic counting.
Most cells appear blue unexpectedly	The sample may have been incubated outside the stated 1-2 minute interval or the culture may contain a high fraction of nonviable cells.	Repeat the count using the specified 1:1 dilution and 1-2 minute incubation so the staining procedure itself is standardized.
Very few blue cells are visible	The mixture may not have been prepared at 1:1 or the incubation may be too short.	Confirm equal volumes of cell suspension and 0.4% Trypan Blue and incubate for the full 1-2 minutes.
Counts differ between replicate hemocytometer loads	The chamber may not have been loaded consistently.	Place the coverslip correctly and load the same freshly prepared 1:1 mixture into the hemocytometer chamber for each replicate.
Counting is delayed after dye mixing	The sample is no longer being evaluated at the datasheet-specified incubation interval.	Standardize counting so microscopy begins after the stated 1-2 minute room-temperature incubation.
Wrong dye-to-cell ratio is used	The sample was diluted differently from the recommended method.	Use a 1:1 ratio of cell suspension to Trypan Blue 0.4% Solution.
The assay is interpreted as staining viable cells blue	The dye-exclusion principle has been reversed.	Interpret blue-stained cells as dead cells and unstained cells as viable cells, as stated in the datasheet.
Storage condition is uncertain	The reagent may not have been kept according to the datasheet.	Store at room temperature.
Safety handling is inadequate	The mutagen warning may not have been considered.	Handle with care and dispose of Trypan Blue according to applicable local regulations, as stated in the datasheet.

Still having trouble? Repeat the count with a fresh 1:1 cell-suspension/Trypan Blue mixture, incubate 1-2 minutes at room temperature, load the hemocytometer consistently, and count promptly. Interpret blue cells as dead and unstained cells as viable.