



LiT Trypan Blue Cell Viability Assay

LiT-TBLU-30mL

Overview

Trypan Blue Solution, 0.4%, is routinely used as a cell stain to assess cell viability using the dye exclusion test. This test is often performed while counting cells with the hemocytometer during routine subculturing, but can be performed any time cell viability needs to be determined quickly and accurately. The dye exclusion test is based upon the concept that viable cells do not take up impermeable dyes (like Trypan Blue), but dead cells are permeable and take up the dye.

Trypan blue protocol

The protocol for trypan blue staining is as follows:

1. Determine the cell density of your cell line suspension using a hemacytometer.
2. Add 10 μL of LiT trypan blue solution, 0.4%, to 10 μL of cells. The ratio for trypan blue staining should remain 1:1—one part trypan blue solution to one part cell suspension.
3. Load a hemacytometer with 10 μL of the cells and trypan blue solution and examine immediately under a microscope at low magnification.
4. Count the number of blue stained cells and the number of total cells. Cell viability should be at least 95% for healthy log-phase cultures.
5. To find the % viable cells = $[1.00 - (\text{Number of blue cells} \div \text{Number of total cells})] \times 100$
6. To calculate the number of viable cells per mL of culture, use the formula: $\text{Number of viable cells} \times 10^4 = \text{cells/mL culture}$. Remember to correct for the dilution factor.