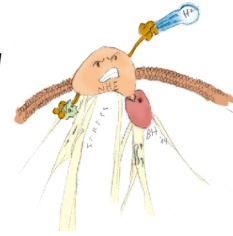


## Quick and Dirty Viable Staining Protocol



Introduction: The real method is attached below and should use a hemocytometer. See Dr. Wallert if you wish to do this. BUT it will take several hours longer this way. Our way is not the “in school method”.

### Calculating the Percentage of Viable Cells

1. Combine 0.5 ml of Trypan Blue Solution and 0.3 ml of PBS.
2. Add 1 ml of the Trypan Blue Mix to each dish of cells, mix well, and incubate for 5 min at room temperature.
3. Count the number of unstained cells under a microscope as described in Section A (Dead cells will take up the Trypan Blue stain).
4. Count the total number of cells.
5. Determine the percentage of viable cells by dividing the number of unstained cells by the total number of cells and multiplying by 100.

The equation is as follows:

$$(\text{Number of unstained cells} / \text{Total number of cells}) \times 100 = \text{Percent Viable Cells}$$

**Comments:** Avoid the exposure of cells to trypan blue for a period longer than 30 minutes. In this case it is possible to observe an increase in the dead cell population (trypan blue positive) due to the trypan toxicity.

**Trypan Blue Solution** Prepare in PBS  
0.4% (w/v) Trypan Blue (0.4 g in 100 ml total)

The correct version is found at: <http://www.bio.com/protocolstools/protocol.jhtml?id=p2151>