National Institute of Environmental Health Sciences

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## Cell Viability Testing with Trypan Blue Exclusion Method

The Trypan Blue dye exclusion test is used to determine the number of viable cells present in a cell suspension. It is based on the principle that live cells possess intact cell membranes that exclude certain dyes, such as trypan blue, Eosin, or propidium, whereas dead cells do not. When a cell suspension is simply mixed with the dye and then visually examined to determine whether cells take up or exclude dye. A viable cell will have a clear cytoplasm whereas a nonviable cell will have a blue cytoplasm.

Periodic cell viability assessment provides an early indicator of the quality of your fresh cells prior to freezing. Viabilities of greater than or equal to 95% are excellent.

### Safety Precautions

Use personal protective equipment when performing this assay, such as gloves and a lab coat. According to the Material Safety Data Sheet (MSDS), trypan blue may cause cancer, so practice appropriate laboratory safety methods.

### Equipment and Supplies

Pipette and tips
Trypan Blue
Hemocytometer and coverslip
Cryovials
Microscope
Counter

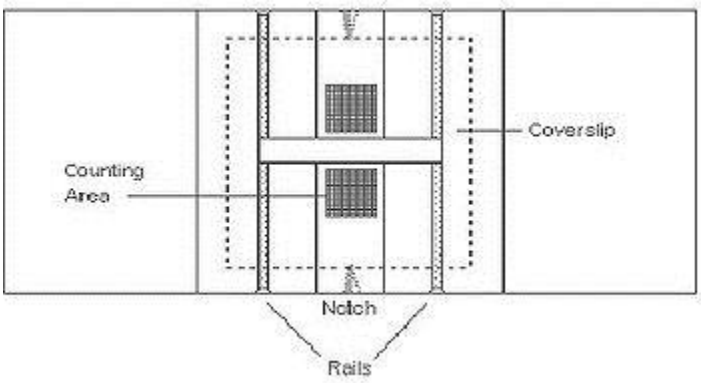
### Procedure

(1) Place 50 µl of cell suspension in a cryo-vial.

(2) Add equal parts of 0.4% trypan blue dye to the cell suspension to obtain a 1 to 2 dilution (example: 50 µl of cells to 50 µl of trypan blue) and mix by pipetting up and down.

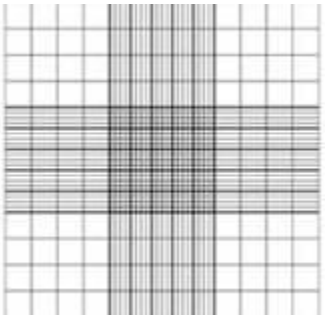
(3) Incubate mixture for less than three minutes at room temperature. If cells are counted after approximately five minutes, viability will be inaccurate due to cell death.

(4) With the cover slip already in place, fill one side of a hemacytometer counter with the cell suspension by placing the tip of the pipette at the notch. Typically, each side will take 10 to 20 µl.




(5) Place the hemacytometer on the stage of a light microscope and focus onto the cells.

(6) Each side of the hemacytometer contains multiple squares. Count all cells (clear and blue) in each large square in each corner of the hemacytometer (see the white areas in the diagram below). Each large square contains 16 small squares. In each large square count cells that are on the border lines on two sides only. Keep track of the number of blue cells separately as well as part of the complete number of cells. Blue cells are the non-viable cells.



(7) Calculate the percentage of viable cells by dividing the number of viable cells by the number of total cells and multiplying by 100 or % viable cells = [1.00 – (Number of blue cells ÷ Number of total cells)] × 100.

(8) Alert your supervisor or lab director if the viability is less than 90%. Document your results on the W drive.