

# Trypan Blue Exclusion Test of Cell Viability

The 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. In this test, a cell suspension is simply mixed with dye and then visually examined to determine whether cells take up or exclude dye. In the protocol presented here, a viable cell will have a clear cytoplasm whereas a nonviable cell will have a blue cytoplasm.

## BASIC PROTOCOL

### Materials

PBS (APPENDIX 2) or serum-free complete medium (APPENDIX 2)

0.4% trypan blue (store in dark bottle and filter after prolonged storage; GIBCO/BRL)

1. Centrifuge an aliquot of cell suspension being tested for viability 5 min at  $100 \times g$  and discard supernatant.

*The size of the aliquot depends on the approximate number of cells present. The aliquot should contain a convenient number of cells to count in a hemacytometer when suspended in 1 ml PBS and then diluted again by mixing with 0.4% trypan blue (e.g.,  $5 \times 10^5$  cells/ml).*

2. Resuspend the cell pellet in 1 ml PBS or serum-free complete medium.

*Serum proteins stain with trypan blue and can produce misleading results. Determinations must be made in serum-free solution.*

3. Mix 1 part of 0.4% trypan blue and 1 part cell suspension (dilution of cells). Allow mixture to incubate ~3 min at room temperature.

*Cells should be counted within 3 to 5 min of mixing with trypan blue, as longer incubation periods will lead to cell death and reduced viability counts.*

*Mixing can be performed in a well of a microtiter plate or a small plastic tube using 10 to 20  $\mu$ l each of cell suspension and trypan blue.*

4. Apply a drop of the trypan blue/cell mixture to a hemacytometer (APPENDIX 3A). Place the hemacytometer on the stage of a binocular microscope and focus on the cells.

5. Count the unstained (viable) and stained (nonviable) cells separately in the hemacytometer. To obtain the total number of viable cells per ml of aliquot, multiply the total number of viable cells by 2 (the dilution factor for trypan blue). To obtain the total number of cells per ml of aliquot, add up the total number of viable and nonviable cells and multiply by 2.

6. Calculate the percentage of viable cells as follows:

$$\text{viable cells (\%)} = \frac{\text{total number of viable cells per ml of aliquot}}{\text{total number of cells per ml of aliquot}} \times 100$$

### COMMENTARY

Dye exclusion is a simple and rapid technique measuring cell viability but it is subject to the problem that viability is being determined indirectly from cell membrane integrity. Thus, it is possible that a cell's viability may have been compromised (as measured by capacity to grow or function) even though its membrane integrity is (at least transiently) maintained. Conversely, cell membrane integrity may be abnormal yet the cell may be able to repair itself and become fully viable. Another potential problem is that because

dye uptake is assessed subjectively, small amounts of dye uptake indicative of cell injury may go unnoticed. In this regard, dye exclusion performed with a fluorescent dye using a fluorescence microscope routinely results in the scoring of more nonviable cells with dye uptake than tests performed with trypan blue using a transmission microscope.

A more sophisticated method of measuring cell viability is to determine the cell's light scatter characteristics or propidium uptake (*UNIT 5.4*). However, this technique is far more time consuming and is necessary only when precise measurements on the number of dead cells in a cell mixture must be obtained.

Trypan blue exclusion, as described in the above protocol, can be performed in 5 to 10 min.

### **Key Reference**

Shapiro, H.M. 1988. *Practical Flow Cytometry*, 2nd ed., p. 129. John Wiley & Sons, New York.

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