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Trypan Blue Exclusion Test of Cell Viability

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Abstract

The protocol described in this Appendix section allows for light microscopic quantitation of cell viability. Cells are suspended in PBS containing trypan blue and then examined to determine the percentage of cells that have clear cytoplasm (viable cells) versus cells that have blue cytoplasm (nonviable cells).

Keywords

Trypan blue; cell viability; dye exclusion

BASIC PROTOCOL

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 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.

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 Å~ 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 Å~ 105 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.

A somewhat higher concentration of Trypan Blue can be used, but this requires some preliminary testing under the conditions being used to determine if it yields better results.

Mixing can be performed in a well of a microtiter plate or a small plastic tube using 10 to 20 μl 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 dye exclusion by flow cytometry. This can be done using typan blue since this protein binds to proteins and then emits a fluorescence signal that can be detected in a flow cytometer (at 660 nm when bound to bovine serum albumin) (See Reference 2 for details for the use of trypan blue in flow cytometry). Alternatively, exclusion can be measured with other light emitting dyes such as propidium iodide (see Unit 5.4 for a description of the use of propridium iodide evaluation of cell viability by flow cytometric analysis). Detailed caparison of estimates of live vs. dead cells using trypan blue exclusion evaluated manually as describe here and evaluated electronically by flow cytometry indicate that the two techniques provide very similar results in experienced hands. Whereas the dye exclusion technique described is more

likely to lead to error because of operator subjectivity, the flow cytometric approach is less suitable for evaluation of cell viability during the performance of complex and time-consuming cell purification techniques. Thus, a flow cytometric technique is applicable only when precise measurements on the number of dead cells in a cell mixture must be obtained.

Recently, an “automated fluorescence microscope viability test” has been reported in which cell viability using propidium iodide in conjunction with a device that counts live and dead cells in a portable microscope cell counter equipped with a microchip. This counter is said to be able to assess cell viability more rapidly than either the manual technique described here or the flow cytometer and to obtain more precise and reliable counts. This device may be useful in situations where many rapidly acquired assays of cell viability are required (3).

It should be noted that cell viability can also be assessed with dyes that bind to DNA (ethidium monoazide), agents that bind to phosphatidylserine (Annexin V) and amine reactive dyes. The use of these alternative agents usually requires flow cytometry and is therefore applicable only under particular conditions.

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

Key Reference

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