Product Data Sheet 748

Trypan Blue 0.4% Solution

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B E A D S ● A B O V E T H E R E S T ™

DESCRIPTION

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.

MATERIAL

Material Supplied

- Trypan Blue 0.4% Solution

Material Required

- Phosphate buffered saline or serum-free complete medium
- Centrifuge
- Hemacytometer

PROCEDURE

1. Centrifuge an aliquot of cell suspension being tested for viability 5 minutes at 100 x G and discard supernatant.
   Note: 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 1mL PBS and then diluted again by mixing with 0.4% trypan blue (e.g., 5 x 10^5 cells / mL).
2. Resuspend the cell pellet in 1mL PBS or serum-free complete medium.
   Note: 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 appoximately 3 minutes at room temperature.
   Note: Cells should be counted within 3-5 minutes of mixing with trypan blue, as longer incubation periods will lead to cell death and reduced viability counts.
4. Apply a drop of the trypan blue / cell mixture to a hemacytometer. 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:

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

7. Trypan blue exclusion, as described in the above protocol, can be performed in 5 - 10 minutes.

COMMENTARY

Dye exclusion is a simple and rapid technique for 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 and propidium iodide uptake. 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.

NOTE

This protocol and resulting text have been excerpted from:
Strober, W. 1997. *Current Protocols in Immunology.* A.3B.1-A.3B.2.

STORAGE AND STABILITY

Keep container tightly closed in a dry, cool and well-ventilated place. Containers which are opened must be carefully resealed and kept upright to prevent leakage. Store at room temperature.

SAFETY

This solution contains sodium azide. Sodium azide may react with lead and copper plumbing to form explosive metal azides. Upon disposal of material, flush with a large volume of water to prevent azide accumulation. Please consult the Safety Data Sheet for more information.

These products are for research use only and are not intended for use in humans or for *in vitro* diagnostic use.

ORDERING INFORMATION

Cat. Code	Description	Size
AA020	Trypan Blue 0.4% Solution	125mL
AA021	Trypan Blue 0.4% Solution	250mL

Order online anytime at **www.bangslabs.com.**