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Chapter 9

Trypan blue dye exclusion assay: Principles, protocols, and applications in cell viability assessment

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1. Introduction:

Cell viability assessment is crucial for determining the number of live cells within a given sample. The Trypan blue dye exclusion test is a widely used method to distinguish between viable and nonviable cells based on their membrane integrity (Davis, 2011). Live cells, which maintain intact cell membranes, can exclude certain dyes like Trypan blue, whereas dead cells cannot. This technique involves mixing a cell suspension with Trypan blue and observing under a microscope: viable cells appear clear, while nonviable cells appear blue. This protocol is particularly valuable in cell culture studies to accurately count live cells before commencing experimental procedures (Butler, 2004; Freshney, 2015).

2. Materials Required:

1. Cell Suspension At 2-6×10^6 Cells/Ml (Macrophages from Peritoneal Cavity of Mouse/Rat).
2. Trypan Blue 0.4% (W/V) In Distilled Water, Pbs.
3. Neubauer Counting Chamber (Hemocytometer).
4. 5 Ml Centrifuge Tubes
5. 10 Ml Syringe with Needle
6. Pasteur Pipette
7. Micropipettes

3. Procedure:

1. Inject 10 mL of PBS into the peritoneal cavity of an anaesthetized mouse/rat (intraperitoneal injection).
2. Massage the abdomen gently for 2-3 minutes.
3. Make a careful incision in the abdominal wall without draining the abdominal fluid.
4. Aspirate the peritoneal fluid with a Pasteur pipette.
5. Euthanize the mouse/rat appropriately.

6. Transfer the peritoneal fluid aspirate to a clean, graduated centrifuge tube.
7. Centrifuge the tube at 1000 rpm for 5 minutes to settle the peritoneal cells.
8. Pour off the supernatant and slowly add fresh 5 mL of PBS.
9. Aspirate the cells and resuspend the sedimented cells gently in PBS using a Pasteur pipette.
10. Centrifuge again at 1000 rpm for 5 minutes and resuspend the cells in PBS.
11. Repeat steps 8 and 9 three times.
12. Lastly, remove the supernatant and resuspend the cell pellet in 5 mL of fresh ice-cold PBS (Adjust the volume of suspension according to pellet size).
13. Pipette 50 µL of the cell suspension into an Eppendorf tube and mix with 50 µL of Trypan Blue (1:1 dilution).
14. Pipette about 100 µL of the Trypan Blue-treated cell suspension onto the hemocytometer using a micropipette.
15. In the case of a glass hemocytometer, fill the two chambers below the coverslip carefully, letting the cell suspension cover the area of counting but not overflow into the moats.
16. In the case of a disposable hemocytometer, pipette the suspension into the well and let capillary action draw it in.
17. Position the grid lines of the hemocytometer under a microscope with a 10X objective.
18. Count the live, unstained cells (those not picking up Trypan Blue) in one group of 16 squares (labeled "L" on Fig. 1). Count cells along square boundary lines on right- or bottom.
19. Count dead, stained cells according to the same rules.
20. Move the hemocytometer to the second group of 16 corner squares and repeat the counting until all four groups of 16 squares at the corners are examined.

4. Observations & Estimation of Viability:

4.1. To determine the number of viable and dead cells/mL:

1. Calculate the average cell count from each set of 16 corner squares.
2. Multiply the average by 10,000 (10^4).
3. Correct for the 1:5 dilution due to Trypan Blue by multiplying the result by 5.
4. The final value represents the number of viable cells/mL in the original cell suspension.

5. Precautions:

1. To avoid possible toxicity to living cells, which could result in an overestimation of dead cells, do not hold the cell suspension and Trypan Blue mixture for more than one to two minutes.

2. Verify that the cell mixture covers the whole counting area by flowing uniformly beneath the coverslip by capillary action.
3. Finish the counting procedure as soon as possible to reduce cell death within the counting chamber, which would otherwise cause the number of dead cells to be overestimated.

6. References:

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