Trypan Blue Viability Assay – Cells in Suspension

Reference

1. Strober W. (2001). Trypan blue exclusion test of cell viability. Current protocols in immunology, Appendix 3, . https://doi.org/10.1002/0471142735.ima03bs21

Abbreviation

1. PBS: Phosphate Buffered Saline

Protocol

1. Centrifuge the cell suspension being tested for 5 min at 100 x g and discard supernatant.
2. Resuspend the cell pellets in 1 mL 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. Sterile filter Trypan Blue solution in order to get rid of particles that would disturb the counting process.
4. Dilute your cell sample in Trypan Blue dye of an acid azo exclusion medium by preparing a 1:1 dilution of the cell suspension using a 0.4% Trypan Blue solution. Non-viable cells will be blue, viable cells will be unstained.
5. Carefully and continuously fill the hemocytometer chamber.
6. Incubate the hemocytometer and cells for 1 – 2 minutes at room temperature. For longer incubations, use a humid chamber. Incubations exceeding 30 minutes may cause decreased cell viability due to Trypan toxicity.
7. Count cells under the microscope in four 1 x 1 mm squares of one chamber and determine the average number of cells per square. For an accurate determination, the total number of cells overlying one 1 mm^2 should be between 20 – 50 cells/square. If the cell density is higher than 200 cells/square, you should dilute your cell suspension.
8. Calculate % viable cells using formula below.

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