Determination of the number of cells and their viability

Trypan blue 0.4% in isotonic phosphate buffer pH 7.4 ⚠

Principle

Trypan blue is actively transported from living cell across the cell membrane. Therefore, when the cell suspension is mixed with a solution of this dye, live cells remain bright, while dead cells are stained blue.

Procedure

1. Add 0.5 ml of trypan blue solution to 0.5 ml of well-mixed cell suspension.
2. After 5 to 15 minutes, the suspension is mixed well and filled into both halves of the Bürker chamber using a 50 µl automatic pipette. It is necessary to apply such an amount of sample that the chamber is just filled, the solution must not overflow into the surrounding grooves.
3. Live (colorless) and dead (blue) cells are counted separately under a light microscope at the lowest magnification. First, 5 squares (4 corner and central) 1×1 mm are counted in one half of the chamber. If the total number of cells is less than 100, the cells in the other 5 squares in the other half of the cell are also counted. The middle line of the triple line is considered to be the boundary of the square. From the cells that lie on the edge of the square, those that even just touch the left or top edge are counted, and vice versa, cells that even just touch the right or bottom edge are not counted.
4. The number of cells is calculated according to the formula $P = \frac{N \cdot D \cdot 1000}{H \cdot S}$, where:
   - P is the number of cells per 1 ml of suspension
   - N is the total number of cells
   - D = 2 (suspension dilution)
   - H = 0.1 (chamber depth in mm)
   - S is the number of squares
5. The percentage of live cells is calculated.

Links

Source

- VEJRAŽKA, M.: Basic techniques of working with tissue cultures. Prague, 2004.