Cell Counting Protocol

Materials

- Fibro Media warm
- Hemacytometer with cover slip
- Eppendorf pipettes
- 0.1% Trypan blue
- Cell suspension
- Microcentrifuge tube
- Microscope

Procedure

1. Warm Fibro Media in water bath.
2. Spray down hood with alcohol.
3. Spray hemacytometer and cover slip with alcohol and allow to air dry.
4. Using appropriate Eppendorf pipettes, combine 450 µl of 0.1% trypan blue and 50 µl of suspended cells in a microcentrifuge tube. Mix well.
5. Inject 50 µl of trypan blue/cell mixture into each side of hemacytometer.
6. Count cells in 5 out of the 9 boxes of the grid using 10x objective with phase contrast. Only two of the four sides of a box should be counted. If a cell is stained blue, then it is dead and should not be counted.
7. Count both sides of the hemacytometer. Add the totals and multiply by 10,000 for amount of cells per ml.
8. Decide how many cells per ml of media are needed. Add appropriate amount of media for desired concentration of cells.