



Northeastern University

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Operating Procedures

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Standard Operating Procedures: Cell Passaging and Operating Procedure

Office of Environmental Health and Safety, Northeastern University

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CELL PASSAGING AND COUNTING PROCEDURE

Summary:

When cells have become confluent, meaning that when you look at them under the microscope, they appear to cover the surface of the whole culture flask, you need to passage them. You need to remove the cells from the flask, count them, and then re-passage them.

References:

Freshney, Culture of Animal Cells handbook, 4th edition.

Required Equipment:

Lab coat
Gloves
Waste media container
Micro-centrifuge tubes (sterile)
Pipettes/pipette tips (sterile)
1x PBS (sterile – Fisher cat# BP24384)
Trypsin/EDTA (Sigma # T4049)
Trypan blue dye (Sigma T8154)
Haemocytometer
Light microscope
Counter
T25/T75 flasks (sterile)
Culturing media (with FBS and anti/antimy added DMEM 1X 10-017-CM))

Procedure – Passaging Cells:

1. Remove the culture flasks from the incubator, and place in laminar flow hood. Tip flask up onto its corner, and remove the media.
2. Rinse twice with sterilized 1x PBS. For T75 flasks use 5mL and for T25 flasks use 3mL. The PBS rinse removes any leftover media from the cells.
3. Add Trypsin/EDTA: this loosens the cells from the bottom of the flask. Use 3mL for T75 flasks, 1.5 mL for T25 flasks.
4. Place flasks in incubator for 5 minutes to allow trypsin to act. When you add it, it will initially be pink color. After incubation, it will be more of an orange/rust color.
5. Remove flasks from incubator, and gently tilt them back and forward once inside the hood. This helps to make sure that all cells are loosened, and that none are being left behind. Turn the flask onto its corner again, and remove trypsin/edta solution, now containing cells, and put into centrifuge tubes. Make sure you label the tubes correctly.
6. Place the tubes into the microcentrifuge, and centrifuge for 10 minutes at 1200rpm. **Make sure you balance the amount of liquids in the tubes, and that you balance the loading of the centrifuge itself.**
7. Remove tubes from centrifuge, placing back into laminar flow hood.
8. You should be able to see the cells clumped together at the bottom of the tube. If you can't you may want to re-centrifuge for a few minutes. Carefully pipette off trypsin/EDTA supernatant.
9. Add 1-1.5mL of media to cell clump, and re-suspend cells by vortexing.

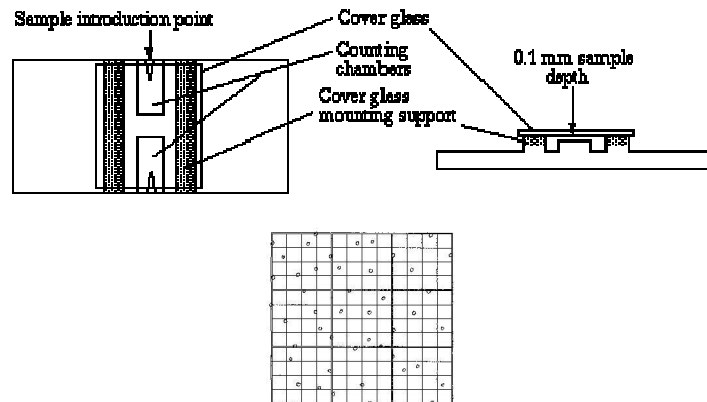
Procedure – Preparing to Count:

1. Take 200µl of your cell suspension, and place in a new 2mL centrifuge tube.
2. Add 20µl of trypan blue, and mix solution with pipettor.

3. Add 10 μ l of trypan/cell solution to hemocytometer.
4. Place cytometer under microscope – use 10X objective.

Procedure – Counting:

1. The counting area is a central square bounded by three lines on all sides. Inside this central square there will be 25 smaller squares, bounded by single lines. Outside this central square there are 8 additional squares of similar dimensions but with less grids. Each square (including the central one) covers a 1mm² area, and its volume equals 0.1 mm³ (see figure below). Count live cells (#live) and dead cells (#dead) separately. Count as many squares as necessary to make for at least 100 cells in each chamber (note the number of squares you used to count). Verify this count by counting the second chamber, and if the measurements agree, proceed to calculate the average count. If the results disagree, clean the hemocytometer with ethanol, dry thoroughly and repeat.



9 squares, each one 1 mm² in area. Grids are not accurate in this cartoon.

2. **CELL DENSITY CALCULATIONS:** The density of the cell suspension (cells/ml) and the cell viability can be calculated as follows:

a. $\rho_{\text{suspension}} =$

$$\frac{\# \text{ Live}}{(\text{number of counted squares}) * (0.1 \text{ mm}^3 / \text{square})} \times \frac{\text{ml. of trypan. blue. and. cell. suspension}}{\text{ml. of. cell. suspension}}$$

b. Cell Viability (%) = $\frac{(\# \text{ live})}{(\# \text{ live} + \# \text{ dead})} \times 100$

Note: If the cells are less than 90% viable, the enzymatic digestion procedure may have been excessively aggressive for them.

3. After determining the cell density in the cell suspension, cells are ready to plate to start the cell culture (see respective protocol).