

Irradiating & Plating MEF's:

Thomson Lab, 2004

1. Remove MEF medium.
2. Wash cells with 5 ml of PBS w/o CaMg (to get rid of trypsin inhibitors).
3. Add 1.5 ml Trypsin/EDTA (0.05% Trypsin) to each flask and allow to sit for about 5 minutes.
4. To loosen cells, either tap flask against the heel of your hand or pipet them off.
5. For every 1 ml of Trypsin/EDTA added, add at least 1 ml of MEF medium to neutralize the trypsin reaction.
6. Add the cell suspension to a 15 ml conical tube and pipet several times to individualize the cells.

7. Perform a cell count:

Mix cell suspension thoroughly and remove 10 ml
Add this to 10 ml Trypan Blue and mix well
Add ≤ 10 ml cell suspension/Trypan Blue mix to hemacytometer

Count bright cells in two of the 4x4 squares--
usually do opposite corners
(ie. A and C, or B and D)

Don't include dead cells in the count--these pick up the Trypan Blue

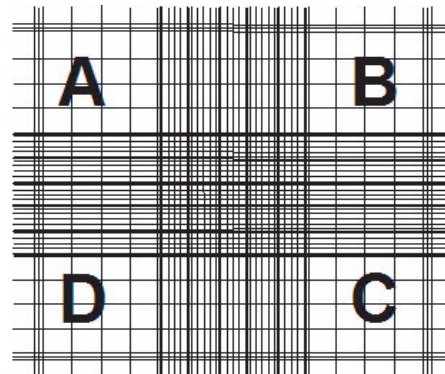
Total cell number = (Total cells counted in two 4x4 squares)

\times (two 4x4 squares) \times (two--Trypan Blue dilution factor) \times (1 x 10⁴--cell dilution factor)
 \times (total ml of cell suspension)

Example: You have just finished counting your sample of 23 ml cell suspension and saw 432 live cells in the two 4x4 squares. Your calculation will look like this:

$432 \text{ cells} \times 2 \times 2 \times (1 \times 10^4) = 4.32 \times 10^6 \text{ cells/ml} \times (23 \text{ ml}) = 99.36 \times 10^6 \text{ total cells}$

8. Irradiate cells for 8000 rads. This number is highly variable between MEF batches--the idea is to irradiate them enough to stop them from growing, but not enough to kill them. We have used between 5000 and 8000 rads in the past.
9. Spin cells at 1000 rpm for 5 minutes.
10. Determine how to dilute cells
Total cell number = (Desired concentration) \times (# ml needed to dilute)
Example: You have 99.36×10^6 total cells, and wish to get your sample to 1.5×10^5 cells/ml.
Your calculation will look like this:
 $(99.36 \times 10^6 \text{ total cells}) = (1.5 \times 10^5 \text{ cells/ml})(x \text{ ml})$
 $x = 662.4 \text{ ml needed}$
11. Resuspend cells appropriately with MEF medium
12. Remove 0.1% gelatin from wells and plate with the following volumes of MEF cell suspension:
0.5ml/well 4 well plate
2.5ml/well 6 well plate and 35mm dish



*NOTES: The general densities for plating MEFs are:
7.5x10⁴ cells/ml for human cells
1.5x10⁵ cells/ml for rhesus cells
2.12x10⁵ cells/ml for conditioned media plates*