Cell Growth on Soft Agar Plates

Buffers Needed:

2x DME (for 250 ml of 2x)

6.7 g Powdered DME
1.85 g sodium bicarbonate
pH to 7.1
Bring volume to 225 ml
Sterile filter and add 0.5 ml pen/strep

2x 3T3 Medium (for 200 ml)

40 ml calf serum (use OLD Gibco serum)
4 ml glutamine
160 ml 2x DME

1.2% agar, sterilized

0.6% agar, sterilized

Procedure:

1. Trypsinize cells and stop trypsinization by addition of serum-containing medium. Make a 1:5 dilution of cells into 2x 3T3 medium. Mix and count the cells.

2. Use 4 x 10^4 cells per well in 6-well dishes. Dilute cells to 4 x 10^4 cells per ml of medium in 2x 3T3 medium. Aliquot into tubes, 1 ml per tube.

3. Add appropriate growth factors (EGF, pertussis toxin etc.) at 2x concentration. (Final volume will be 2 ml per well.

4. Heat agar in microwave until melted. Place in 50˚ C water bath to cool. It is ready when you can hold the bottle in your hand without pain.

5. For bottom layer, mix 1 ml 2x 3T3 medium with 1 ml 1.2% agar per well. Aliquot into wells and let harden.

6. For top layer, add 1 ml 0.6% agar to each tube of cells, media and additions. Cap and mix quickly. Pour into wells. Rock to spread out agar and let cool. Place at 37˚ C.

7. After 10 to 14 days count the number of colonies in ten random fields. Usually count colonies as anything larger than the cross hairs on the microscope (approx. 16 cells)