Collagen Gel Colony Formation Assay

(Hue Luu, 01-24-2007)

Materials:

1) Type I Collagen (3.856 mg/ml Collaborative Biomedical Sciences Cat#40236). Concentrations may vary depending on batch.
2) 24 or 12-well plates.
3) 2x media including all if its components (if you need to add induction drugs such as Dox, make sure it is 2x).

Bottom Layer:

1) Need 150ul of solution per well (includes media, collagen, and bicarb) for 24 well plate. Prepare 3 ml total for a 24 well plate. The recipe below makes 4.5 ml which can be made smaller to save reagents. Prepare in a 5 ml tube on ice.

2x media	1.8 ml
Collagen (on ice)	2.25 ml
7.5% NaHCO3	0.45 ml
	4.5 ml

2) Quickly add 150 ul of the mix to each well and spread evenly (esp. the edges of the wells). Avoid air bubbles by gentle pipeting.
3) Let solidify at 37 degrees C for > 5 mins.

Top layer

1) You will need 160 ul per well
2) Prepare this layer separately for each clone/condition tested. I usually trypsinize and count my cells while the bottom layer is hardening. Keep cells in suspension on ice and periodically swirl.
3) In microfuge tubes, prepare the following for triplicate wells.

2x media	320 ul
Collagen (on ice)	350 ul
Cells	50 ul
7.5% NaHCO3	80 ul
	800 ul

4) After the bicarb, pipet quickly up and down with a P1000 to mix well. Add 160 ul to each well and gently swirl to coat evenly. Avoid air bubbles.
5) Final conc. of media is 0.8x and 1.69 mg/ml collagen.
6) Note: Be sure to add bicarb immediately after adding cells because collagen is acidic….which can be harmful to your cells. The amount of cells per well vary depending on the cell line and conditions. I start with 8,000 cells per well in a 24 well plate.
7) Let solidfy at 37 degrees C for > 5 mins
8) Add 1x media to keep gel from drying out.
9) Let media equilibrate in the incubator for several hours and then wrap plates and incubate for desired duration of experiment (7-14 days).