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Soft Agar Colony Formation Assay

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Overview: This assay is designed to assay a cell's ability to grow unattached to a surface and therefore suspended in agar. The assays are done in 6cm plates. A bottom layer of enriched media + agar is poured first (2.5ml), after solidifying this is followed by a layer containing a lower amount of agar and containing a specified number of cells (cell layer = 5ml), after solidifying a top layer is poured. The plates are placed in the incubator and after two weeks or so, colonies are counted by naked eye.

1. Make sol'ns of 2X Iscove's Media (Gibco BRL, dissolve one 1L packet in 500ml of water) and filter sterilize, make about 100 ml of 1X by diluting the 2X, separate from this 100ml make 100 ml of 1X Iscove's containing 10% FBS
2. Make Noble Agar (Difco) at 1.3% and place in 55° C water bath to keep in liquid phase
3. Determine the number of sample pairs you will be making (a sample pair consists of one plate control and one plate experimental. I usually made 4 sample pairs or an n of 4. Add one to this, so n of 4 +1 = 5
4. The bottom and top layer solutions are made as one and divided in half. The cell layer solution is made separately.
5. Determine the volume of reagents that make up each of the above solutions according to the following formula:

   Bottom/Top layer - 2X Iscove's 5ml X n sample pairs (5) = 25 ml
   FBS - 1ml X n sample pairs (5) = 5ml
   100X Glutamine .1 ml X n sample pairs = 0.5 ml
   1.3% Noble Agar - 5ml X n sample pairs = 25ml
   pour these volumes into a 50 ml conical and separate the total volume in 1/2 into one tube for the bottom and the other for the top

   **Do Not add the Agar until you are just ready to pour, so separate the total (without the agar) into a bottom and top tubes (the volume of agar that will be added to each will be 12.5ml)

   Cell Layer media - 2X Iscove's 2 X n = 10ml
   1X Iscove's 4 X n = 20ml
   FBS 1 X n = 5ml
   100X glutamine 0.08 x n = .4 ml
   Noble agar 2 X n = 10ml (again do not add this until ready to pour)
6. Pour 12.5 ml of 1.3% Noble agar to the bottom layer tube, mix thoroughly and pipette 2.5 ml to each of 8 plates, place in 37° C incubator

7. Trypsinize cells, collect disattached cells and spin down, rususpend pellet in 5ml of 1X Iscove's + 10% FBS for counting. Determine the conc of cells in cells/ml and then calculate the volume of media that will yield 2 X 10^4 cells (one sample pair or n = 2 X 10^4 cells, or 1 X 10^4 cells/ 6cm plate)
8. Add volume for 2 X 104 cells in a 15 ml Falcon tube containing 1 ml of 1x Iscove's media.
   Do this for n samples (in this case 4 Falcon tubes, 2 control, 2 experimental)
9. Add the volume of agar calculated for the cell layer to the tube containing the cell layer solution and mix thoroughly and add 9ml of the cell layer solution to each of the four Falcon tubes and mix thoroughly.
10. Remove plates from incubator (bottom layer has solidified at this point) and add 5ml from each Falcon tube to each plate and allow to solidify for 30 min at RT.
11. Add the last 12.5 ml of agar to the tube containing the top solution, mix throughly and pipette 2.5ml to each plate.
12. Place plates in incubator and remove when colonies can be seen with naked eye, (timing depends of the tumorgenicity of the cell line but typically is around two weeks.
13. To count colonies, take a digital picture of each 6cm plate by placing the plate on a light box and using a digital gel photography set up. Print out a picture of each plate and manually count the number of colonies per plate. Determine the average number of colonies per plate for both control and experimental groups