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Anchorage-independent growth assay or Soft Agar assay

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- 1 Prepare 5% SeaKem low gelling agarose in distilled water and autoclave it.
- 2 Warm 10% serum media in 48 °C water bath
- 3 Melt the agar in microwave for some minutes. Be sure to mark the level of agar prior to heating so the level can be brought back up with distilled water, to maintain the correct concentration of agar.
- 4 Allow the agar to cool at room temperature for 10 minutes, then place it in the 48 °C water bath.
- 5 Make a dilution of agar in prewarmed media for a final concentration of 0.6% agar and considering to pour 4 ml in each p60 plastic plates.
- 6 Pour the agar in plates and let them sit at room temperature for 40 minutes to solidify, then place in the 37 °C CO2 incubator to allow the plates to adjust to the proper pH.
- 7 Prepare a dilution of agar in prewarmed media for a final concentration of 0.3% agar and considering to pour 3 ml in each p60 plastic plates.
- 8 Trypsinize cells and resuspend in a minimal volume of media to count them and make the desired cell dilution.
- 9 Add 3 ml of 0.3% agar into appropriate number of conical tubes.
- 10 Add the right amount of cells to each tube and, working quickly, gently shake the tube in order to suspend the cells evenly through the agar.
- 11 Pour the entire content of tube on top of a previously poured 0.6% solidified agar plate.

- 12 Swirl plates gently and let them sit at room temperature for 40 minutes to solidify, then place in the 37 °C CO₂ incubator.
- 13 After 24 hours, feed cells with 1 ml of 10% serum media and check weekly that they are well hydrated.