MAY 14, 2020

Anchorage-independent growth assay or Soft Agar assay

PLOS One

Marzia Ognibene¹

¹Laboratorio Cellule Staminali Post Natali e Terapie Cellulari, IRCCS Istituto Gaslini, Genova, Italy

Marzia Ognibene

OPEN ACCESS

DOI:

dx.doi.org/10.17504/protocols.io.bgeajtae

External link:

<https://doi.org/10.1371/journal.pone.0244069>

Protocol Citation: Marzia Ognibene 2020. Anchorage-independent growth assay or Soft Agar assay. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.bgeajtae>

MANUSCRIPT CITATION:

Ognibene M, Pezzolo A (2020) Ezrin interacts with the tumor suppressor CHL1 and promotes neuronal differentiation of human neuroblastoma. PLoS ONE 15(12): e0244069. doi: [10.1371/journal.pone.0244069](https://doi.org/10.1371/journal.pone.0244069)

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working
We use this protocol and it's working

Created: May 14, 2020

Last Modified: May 14, 2020

PROTOCOL integer ID:
37026

- 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.