Colony formation assay in Matrigel

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Jun 28, 2022

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dx.doi.org/10.17504/protocols.io.q26g747k3gwz/v1

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ABSTRACT

This is a colony formation assay that I have set up as an alternative to soft-agar assay. It is based on a 3D on-top assay (Lee et al. 2007, 4, 4, 359) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2933182/

ATTACHMENTS

Lee et al. Three-dimensional culture models of normal and malignant breast epithelial cells.pdf

DOI

dx.doi.org/10.17504/protocols.io.q26g747k3gwz/v1

PROTOCOL CITATION

Goran Tomic 2022. Colony formation assay in Matrigel. **protocols.io** https://dx.doi.org/10.17504/protocols.io.q26g747k3gwz/v1

KEYWORDS

Matrigel, colony formation

CREATED

Jun 28, 2022

LAST MODIFIED

Jun 28, 2022

PROTOCOL INTEGER ID

65555

1 Thaw Matrigel on ice at 4 C overnight. Prechill one 48-well plate, 200 uL box of tips.

2 Coat prechilled culture surface with a thin layer of Matrigel (80 uL per well in a 48-well plate)
Incubate for 20 min at 37C to allow to gel

3 Trypsinise cells (Filter through a 40 um mesh to make single cells)
Aliquot cells in a 1.5 mL eppendorf tube to have 2500 cells/100 uL, multiplied by the number of replicates, and spin down at 1200 rpm. Depending on the cell line, optimisation of cell number might be needed.

4 Add 100 uL of cell suspension on top of Matrigel in wells.
Leave for 20 min at 37C for cells to attach to Matrigel.

5 Chill the remaining medium on ice and add Matrigel to 10%
Gently add 100 uL of the 10% Matrigel medium on top

6 Maintain culture for 4 days. Change the medium every 2 days (with 10% Matrigel)

7 Observe the formation of colonies. Once ready for imaging, scan on Oxford Optronics colony counter or another method of choice. https://www.oxford-optronix.com/gelcount-cell-colony-counter

It helps to top up the wells with PBS or medium to reduce the liquid meniscus interfering with the scanning.

Citation: Goran Tomic Colony formation assay in Matrigel https://dx.doi.org/10.17504/protocols.io.q26g747k3gwz/v1