Soft-Agar Assay

Xin Chen Lab modified by Tao Su 2019

Materials

Agar (Difco Noble Agar, BD Bioscience cat # 214220)
PBS
DMEM with 20% FBS and Pen/Strep
6-well plate
Heating block with dry beads and water bath

Prepare 3% agar in PBS

Dissolve 1.5 g agar powder in 50 ml PBS in a 100 ml glass bottle.
Autoclave the solution and store at 4°C, color looks a little pinkish.
Melt 3% agar in microwave oven before use and cool down to 50°C.

Making 1% agar in DMEM with 20% FBS and P/S

10 ml 3% agar in PBS
20 ml DMEM 20% FBS P/S
Remain the solution at 50°C to prevent gelling.

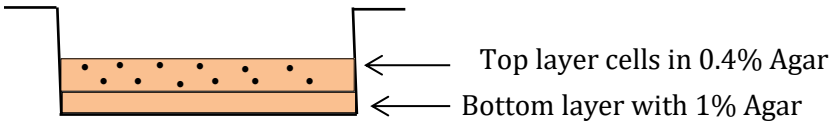
Prepare bottom layer of gel with 1% agar

Add 1% agar 1.5 ml or 2 ml/well in 6-well plate.
Leave the plate at RT for 30 min to gel. During this time to trypsinize cells.

Prepare cells: 40,000 cells/2 ml culture medium with 0.4% agar/well

Procedures:

Prepare cells for 2 wells.
Suspend 1 x 10^5 cells in 3 ml pre-warmed DMEM with 20% FBS and P/S.
Add 2 ml of 1% agar in a 50 ml tube **keep at 50°C** before mixing to prevent gelling.
Mix the cells with 1% agar gently, the cell density should be 20,000/ml and place 2 ml the mixture on the bottom layer of agar with care to avoid bubbles, leave the plate at 4°C for 10 min allowing quick gelling.
Add 0.5 ml culture medium on top of the agar gel.
Incubate cells at incubator with 5% CO_2 at 37°C, change medium every 4 days. Cells form colonies after 7 to 10 days. Colonies can remain alive for over 4 weeks.

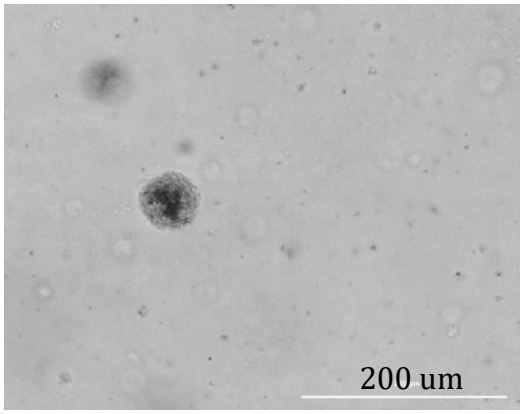


Stain and count cell colonies

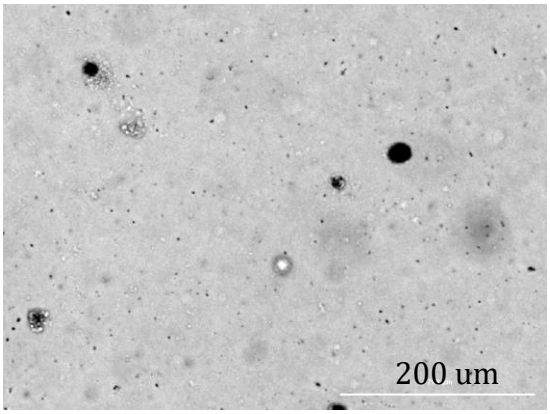
Stain plates with 0.5 ml of 0.005% Crystal Violet for no less than 1 hour.
Take pictures and count colonies using a dissecting microscope.

Cells form colonies in soft agar after 2 to 3 weeks. Microphotographs of live cell colonies by invert microscope.

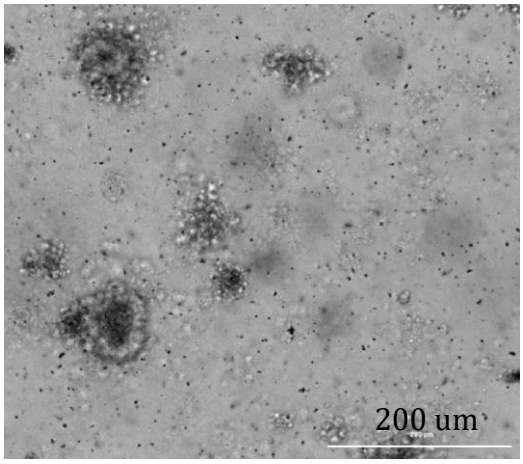
Mouse HCC3



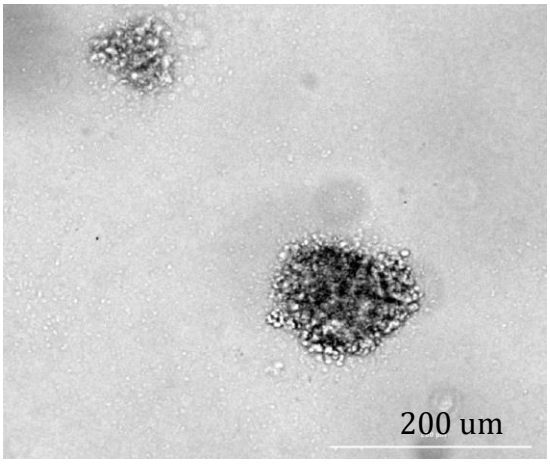
Mouse DW001 cMyc/MCL1



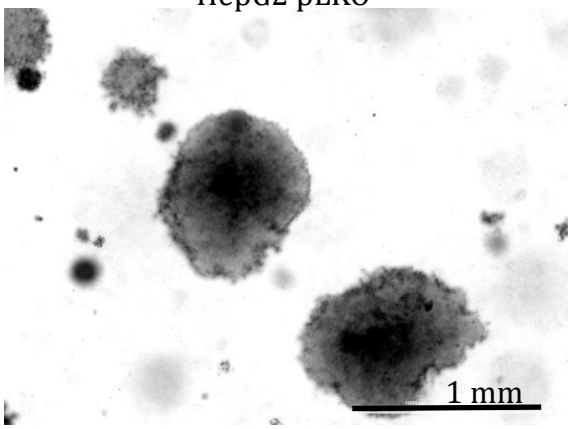
Mouse HCC4



Human 1023-5 iHep cMyc/MCL1



HepG2 pLKO



HepG2 shRNA Rictor