Lazzara Lab
Nisha G. Sosale
Modified by Evan Day, June 8, 2017

Spheroid Protocol

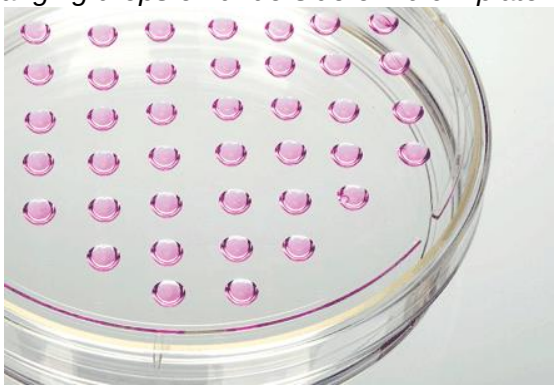
Materials

Component	Vendor	Catalog #
MEM, 10x	Gibco	11430-030
Glutamine, 200 mM	Gibco	25030-081
Fetal Bovine Serum	Seradym	97068-085 218B14
Sodium Bicarbonate ($NaHCO_3$, 7.5%)	Gibco	25080-094
1x Dulbecco's PBS (DPBS)	Gibco	14190-144
Bovine Collagen I (5 mg/mL)	Cultrex	3442-050-01

Hanging Drop Culture

1. Trypsinize cells, and perform cell count.
2. Resuspend ~$2.5x10^5$ cells in 875 µL media. The suspension density can be adjusted as needed. Generally, a range of 5,000-15,000 cells per 30 µL drop (~$1.5x10^5$ - $4.5x10^5$ in 875 µL total) works well.
3. Dispense 25 × 30 µL of the cell suspension to the underside of a 10 cm plate lid.
4. Dispense 5-10 mL PBS into the bottom of the plate to prevent hanging drops from evaporating.
5. Carefully invert the lid and place it on top of the plate bottom. Transfer plate to incubator for 1-4 days.

Hanging drops on underside of 10 cm plate lid



Embedding – For preparation of 6 gels

1. Prepare collagen mixture according to recipe below, and store collagen on ice to prevent solidification.

Component	Volume (µL)
10x MEM	420
Glutamine	38
FBS	462
$NaHCO_3$	100
1x DPBS	2100
Collagen (5mg/mL)	1400
Total	~4500

2. Ensure that mixture has a "red color", and adjust sodium bicarbonate as needed to achieve this.
3. Dispense 300 µL of collagen mixture to 6 wells in a 24-well plate.
4. Incubate ~30 min at room temp to solidify.

5. Transfer hanging drop mixture to 15 mL conical tube. Allow spheroids to settle for 1-2 min or longer to allow for spheroids to visibly settle. Allowing spheroids to settle for too long will lead to carry over of single cells and smaller aggregates into the gels.
6. Once the first layer of collagen is solidified, aspirate media off the settled spheroids. Then re-suspend the spheroids in 300 µL cold collagen mixture per well (1800 µL for a total of 6 wells).
7. Dispense 300 µL of the collagen-spheroid mixture per well.
8. Incubate ~30 min at room temperature to allow to solidify.
9. Once solidified, add 0.5 mL complete media to each well. Inhibitor or growth factors can be added to this media when appropriate.
10. Change media every 2-3 days by carefully aspirating media on top of the gels and replacing.

PD3077 (murine PDAC cells) spheroids embedded in collagen gels