**Spheroid Culture**

**Materials**

**Reagents**

Complete media

Sterile PBS

Trypsin

Ice

Poly-NIPAM gel

Trypan Blue

**Plastics/ glassware**

24 well plate

15 mL tube (2)

1.5 mL Eppendorf tube

P-1000 positive displacement pipette

Large beaker

Cell counting slide

**Cell culture procedure**

1. Grow cells in 2D culture until confluent
2. Aspirate media from flask and rinse cells twice with sterile PBS
3. Add Trypsin to flask, gently agitating cells to ensure contact is made with Trypsin
4. Place flask in incubator for 5 minutes, or until cells are sufficiently removed from plate; however, do not exceed 20 minutes, as Trypsin is cytotoxic
5. After Trypsinization, add media to Trypsin in a 2:1 ratio
6. Transfer cells suspended in the Trypsin/ media solution to a 15 mL tube and spin in centrifuge for 5 minutes at 200 g

**Spheroid procedure**

1. During this time, label and place a new 6 well plate into the incubator
2. Add ice to a beaker and place Poly-NIPAM gel flask and a 15 mL tube on ice
3. Aspirate Trypsin/ media solution from 15 mL tube and resuspend cells in 1 mLs media (cells should be greater than 1 million/ml)
4. Using a 1.5 mL tube, add 20 uLs of cell suspension
5. Subsequently add 20 uLs of Trypan blue and resuspend to mix with cells thoroughly
6. Add 20 uLs of this solution to a cell counting slide and calculate live cell population
7. Calculate number of cells needed to achieve 15,000 cells per gel in a 150 uL volume of Poly-NIPAM gel (1 x 105 cell/ml) (This cell density works well for some cell lines: B.C: SkBR3, AU565, BrM2a, BT549, HCC202, HCC1395, HCC1419, HCC1806, HCC1419, HCC1428, HCC1806, LM2, MCF7, MDA-MB-134, MDA-MB-175, MDA-MB-361, ZR-75-1; O.C: OVCAR-3, SKOV-3; P.C: PC3). Make 3 gels per well

Example: If you need to prepare 12 gel: Multiply 150 \* 12 – 1800 ul=1.8 ml

 If you live cell population was 3.57 x 106 cells/ml

$$C\_{1}V\_{1}=C\_{2}V\_{2}$$

$$3.57 x 10^{6}\*V\_{1}=1 x 10^{5}\*1.8 ml$$

$$V\_{1}=0.05 ml$$

We need 50 ul of cells in 1.8 ml of Poly-NIPAM gel

1. Remove 6 well plate from incubator
2. Aliquot 1.8 ml of Poly-NIPAM to the chilled 15 mL tube using a positive displacement pipette
3. Add appropriate amount of cells (50 ul-using calculation above) to gel and stir with tip (do not resuspend with this tip as the gel is very viscous)
4. Add 150 uLs of cell/ Poly-NIPAM suspension to one well in the 6 well plate using positive displacement pipette
5. Hold pipette as vertical as possible; try not to add any bubbles to the gel by pipetting at a steady pace both when taking the suspension from the 15 mL tube and when placing the gel in the well
6. Once all the gels have been pipetted into their wells, place plate in incubator for 5 minutes
7. When ready, add 3 mL of media to each well and return to incubator to allow spheroid formation