**Spheroid Recovery Protocol**

**Materials**

**Reagents**

Cold Serum Free Media (SFM)

Ice

**Plastics/ glassware**

15 mL tube

Large plastic container to hold ice

Cut tips

**Spheroids recovery for lysing samples**

1. Grow cells in 3D culture, between 1-2 weeks (Depends on cell line)
2. Aspirate old media from 6 well plate
3. Add 3 mL of cold SFM per well
4. Place plate on ice for 5 minutes, to dissolve the Poly-NIPAM gel, until dissolved.
5. Transfer spheroids to a 15 mL tube
6. Add 3-5 mL of cold SFM per tube and spin in centrifuge for 5 minutes at 200 g
7. Remove supernatant and add lysis buffer.

**Spheroids recovery for 3D Hydrogels**

1. Follow step 1-5 above
2. Add 3 mL of cold SFM per tube and incubate for 30 on ice to pellet spheroids
3. Gently remove the supernatant
4. Suspend spheroids in PEG-MAL (see protocol for 3D PEG-MAL Gels-Use cut tips), or natural hydrogels of interest. For PEG-MAL gels, transferring spheroids from 1 polyNIPAAM gel into 9 10uL PEG-MAL gels works well for cell lines such as MDA-MB-231 or SkBr3.