Harvesting Protein Lysates from 3-D Acinar Cultures
(for quantification of non-phosphorylated, intracellular, stable proteins)

1) Aspirate the media from the wells for harvesting.
2) Add high concentration trypsin (0.25%) for washing and remove immediately. For a single well of an 8-well chamber slide, use 250 μl of trypsin for washing. For cultures grown in larger wells, such as 24-well or 12-well plates, use 500 μl or 1 ml, respectively for washing.
3) Add half of the volume of high concentration trypsin used in step 2 to each well to trypsinize the Matrigel™ (125 μl trypsin for an 8-well slide, 250 μl for a 24-well plate, and 500 μl for a 12-well plate).
4) Immediately scrape the well to remove the cells and matrigel. For this step, we use a sterile plunger from a 1 ml syringe to scratch the bottom of the well.
5) Resuspend the mixture of trypsin, Matrigel™, and cells several times using a P1000 to break up the Matrigel™.
6) Collect the trypsin, Matrigel™, and cells in a 15 ml conical tube.
7) Repeat steps 3-6 using a fresh volume of trypsin to remove any remaining Matrigel™ from the well and collect the mixture in the same 15 ml conical tube.
8) Incubate the trypsin, Matrigel™, and cells for 20-30 minutes at 37°C.
9) Spin the cells at 150x*g* in a tissue culture centrifuge for 3 min.
10) Resuspend the cell pellet in 5 mls of Resuspension Medium to quench the trypsin (See Media Table for recipe).
11) Spin the cells at 150x*g* in a tissue culture centrifuge for 3 min.
12) Resuspend the cell pellet in 1 ml of PBS and transfer to an eppendorf tube.
13) Spin the cells for 3 minutes at 2000 rpm in a microcentrifuge. Aspirate the PBS from the cell pellet. (The cell pellet can be flash frozen in liquid nitrogen and stored at –80°C, if necessary. For lysates harvested over several different days, we freeze the pellets on individual days and lyse the cells once all days have been harvested).
14) Resuspend pellet in RIPA or NP40 lysis buffer supplemented with protease inhibitors (approximately 100 μl/4 wells of a chamber slide).
15) Incubate lysate on ice for 15-30 minutes.
16) Spin the lysates for 15-20 minutes at 4°C at 14,000xg (maximum speed) in a microcentrifuge to clear the lysates.
17) Collect supernatant in new eppendorf tube, and flash freeze the lysate in liquid nitrogen.
18) Store at –80°C. Standard BCA or Bradford Assays can be used to quantitate protein levels prior to immunoblotting.