Harvesting Protein Lysates from 3-D Acinar Cultures
(for quantification of phospho-proteins, extracellular proteins, or quickly degraded proteins)

1) Aspirate the media from the wells for harvesting.
2) Wash briefly with 4°C PBS supplemented with protease inhibitors (phenylmethylsulfonyl fluoride [10 g/ml], leupeptin [1 g/ml], aprotinin [1 g/ml], pepstatin [1 g/ml]). Use 250 μl per well for an 8-well chamber slide, 500 μl per well for a 24-well plate, and 1 ml for a 12-well plate.
3) Lyse the cells directly in each well by adding RIPA or NP40 buffer supplemented with protease inhibitors, and incubate for 15 minutes at 4°C.
4) Collect the Matrigel and acini in lysis buffer and pull through a 27-gauge needle 3-5 times.
5) Incubate at 4°C for 15 minutes.
6) Spin the lysates for 15 minutes at 4°C at 14,000xg (maximum speed) in a microcentrifuge to clear the lysates.
7) Collect supernatant in new eppendorf tube, and flash freeze the lysate in liquid nitrogen.
8) Store at –80°C. Standard BCA or Bradford Assays can NOT be used to quantitate protein levels prior to immunoblotting due to the large amount of proteins present in the Matrigel™. The CytoTox 96 Assay from Promega can be used on these lysates to measure lactate dehydrogenase (LDH) levels, and lysate normalization can be performed prior to immunoblotting.