Mammary epithelial cell (MEC) isolation for whole cell RIPA extracts

MEC Isolation:

- Remove #3 and #4 mammary glands and place in pre-weighed 50 ml conical containing cold F12 media.
- Determine the weight of the tissue and aliquot 10 ml/g of digestion buffer to 50 ml tubes.
- Mince the mammary gland tissue in a 10-cm plate using two scalpels, until the pieces are <1mm.
- Place the minced tissue in digestion buffer and incubate at 37ºC with shaking at ~125 rpm for 1-2 hrs, giving the tube a hard shake every 30-45 minutes.
- After digestion, bring the volume up to 35 ml with F12 media.
- Spin 3 min at 800 rpm in the clinical centrifuge at 4ºC.
- Transfer the supernatant to a clean tube and save the pellet.
- Spin the supernatant 10 min at 1500 rpm in the clinical centrifuge at 4ºC.
- Discard the supernatant and combine the two pellets with 10 ml of F12 media, placed in a fresh tube.
- Bring the volume up to 35 ml and spin 5 min at 1000 rpm.
- Discard the supernatant, and repeat wash process 2-3 more times (until media is clear).

RIPA Protein Extraction:

- Resuspend the pellets in RIPA buffer + inhibitors (volume depends on size of pellet).
- Vortex at the highest setting for 1 minute.
- Place tube on ice for 30 minutes.
- Spin 10 min at 16,000xg at 4ºC.
- Transfer the supernatant to a new tube and discard the pellet.
- Spin the supernatant for 10 min at 16,000xg at 4ºC.
- Transfer the supernatant to a new tube and discard the pellet. The cleared supernatant is the whole cell extract.
- Quantitate the protein concentration by BCA assay (Pierce).
- Aliquot the extract in 0.5 ml tubes and store at -80ºC.

Note: This protocol can also be used to make nuclear and cytoplamic extracts from the enriched MEC pellet, using the Pierce kit (NE-PER, #78833).

Digestion buffer:	20 ml F12 media		
	40 mg collagenase (=> 2 mg/ml)		
	1.5 mg Hyaluronidase (=> 100U/ml @ 1320 U/mg)		
RIPA buffer:	37.25 ml water		
	5 ml	10x PBS	=> 1X
	5 ml	10% NP-40	=> 1%
	2.5 ml	10% deoxycholate	=> 0.5%
	250 µl	20% SDS	=> 0.1%
RIPA buffer + Inhibitors	5 ml	RIPA buffer	
	5 µl	NaF	[1 M]
	2.5 µl	PMSF	[0.2 M] in ethanol
	0.5 µl	Okadaic Acid	[10 µM]
	5 µl	Sodium vanadate	[0.1 M]
	1 µl	Sodium molybdate	[0.5 M]
	1/2 tablet	Roche protease inhibitor cocktail tablets, EDTA-free (#1836170)	