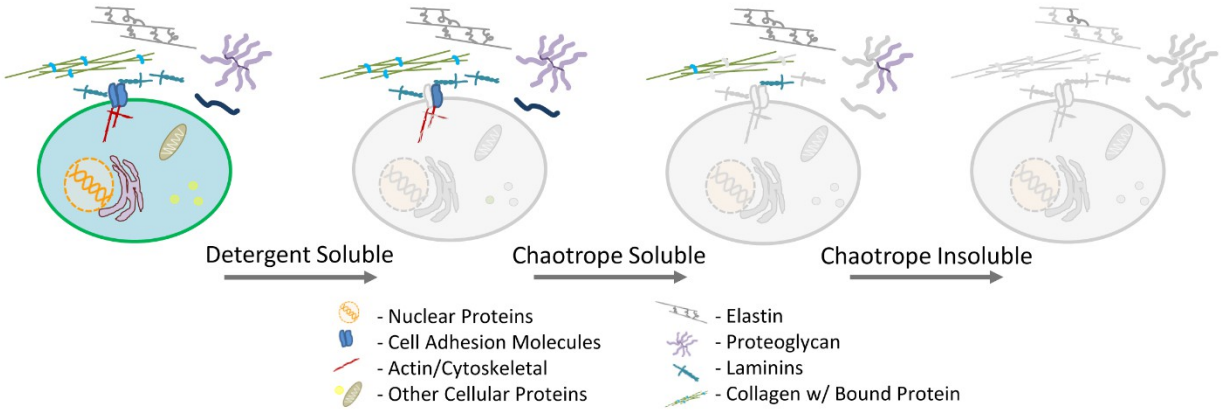
Method for ECM Protein Extraction from Tissue



1) Pulverize tissue in liquid nitrogen
2) Homogenize tissue in detergent extraction buffer with BeadBeater or alternate homogenization tool.
3) Centrifuge sample at high speed, remove supernatant (Detergent Soluble Fraction) and proceed with protocol on remaining pellet.
4) Add additional detergent extraction buffer to pellet, vortex, spin, and combine supernatant with detergent soluble fraction from Step 3.
    a. Repeat 1x.
5) Vortex remaining pellet with chaotrope extraction buffer, spin, and remove supernatant (Chaotrope Soluble Fraction). Proceed to step 6 with remaining pellet.
    a. Repeat 1x.
6) Chemically digest remaining pellet in acidic environment for at least 18 hours.
7) Neutralize and wash pellet, solubilize in buffer, spin, remove supernatant (Chaotrope Insoluble Fraction).
    a. Remaining pellet should contain less than 1% of total protein.
8) Proceed with digestion procedure -- We recommend FASP or GeLC-MS/MS for detergent/chaotrope removal.
    a. Optional – Spike in Stable Isotope Label Peptides, QconCATs or AQUA, before or after digestion procedure, respectively, for downstream targeted MS analysis.
9) Extract Peptides and proceed with LC-MS/MS analysis for global protein identification or LC-SRM analysis for targeted protein ID and quantification.