STANDARD OPERATING PROCEDURE	**SOP No. 008** **Protein Extraction from Frozen Tissue**
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Electronic Filename: PCBN.SOP08.v1.0 Protein Extraction Frozen Tissues	

1. PURPOSE

To describe the procedure for the extraction of protein from frozen tissue. NOTE: This SOP does not cover detailed procedures for handling Human Biological Materials or hazardous chemicals and it is recommended that personnel following this SOP refer to institutional safety guidelines.

The following SOP for protein extraction from frozen tissue is followed by the JHU Network Site of the PCBN.

2. RESPONSIBILITIES

Authorized personnel extracting protein from frozen prostate tissue must ensure that:

- all procedures are followed correctly
- all samples are adequately coded during processing
- all documentation is completed, and accurate records maintained on all samples

3. HEALTH AND SAFETY

Personnel carrying out this procedure must maintain safe working practices and observe all relevant Environmental Health and Safety (EH&S) guidelines. This includes the appropriate use of Personal Protective Equipment (PPE), and the procedures for waste disposal, disinfection & spill clean-up and biosafety.

4. EQUIPMENT AND MATERIALS

Equipment	Materials
Cryostat, chuck, freezing bars	Sodium deoxycholate (1.1%)
	Cryovials
	Microcentrifuge tubes
	5M NaCl
	20% SDS
	1M Tris-HCl (pH 7.5)
	Triton X
Refrigerated Microcentrifuge	OCT
Vortex	Protease Inhibitor Cocktail (Sigma P8340)
	RNase-free water
	Sterile blades for cryostat
	Phosphatase Inhibitor Cocktail (Cell Sig. 5870)

5. PROCEDURES

5.1 Sample Preparation

- Collect tissue samples for protein extraction from -80°C storage and place on dry ice.
- Cut 10 µm sections in a cryostat at -30°C.

  NOTE: The tissue samples may require re-embedding in OCT, before 10 µm sections are cut.

  For each sample, a section is harvested at maximum diameter location at thickness of 5 µm both immediately before and immediately after the tissue sections collected in the tube for H&E staining and stored in the biorepository.

- Collect sections into 3 ml cryovials (per sample).

5.2 Protein Extraction

- Make appropriate amount of RIPA buffer (~400 µl per 50, 10 µm tissue sections)
- Recipe for 10 ml RIPA buffer:
  - 9 ml sodium deoxycholate (1.1%)
  - 0.25 ml of 1M Tris-HCL (pH 7.5)
  - 0.3 ml 5M NaCl
  - 0.1 ml Triton X
  - 50 µl 20% SDS
  - 300 µl sterile water

  - Protease Inhibitor Cocktail (Sigma P8340) diluted 1:100 - Add just before Use

  - Phosphatase Inhibitor Cocktail (Cell Signaling 5870) diluted 1:100 - Add just before Use

- Pipet appropriate amount of ice cold RIPA buffer + inhibitors to each sample and vortex.
- Place samples on ice for 20 minutes, vortexing briefly every 5 minutes.
- Centrifuge for 10 minutes at 14,000 rcf at 4°C.
- Transfer supernatant, leaving pellet undisturbed, to a new tube

5.3 Protein Quantification- BCA assay

- Make appropriate amount (200 µl per well) of Pierce BSA Protein Assay Reagent A and B mix (50:1 mix of Reagent A (cat. 23228) and Reagent B (cat. 23224)). Invert to mix well.
- Load 10 µl of each Pierce™ Bovine Serum Albumin Standard (cat. 23208) in duplicate into a 96-well flat bottom clear plate.
- Dilute protein samples 1:20 in RIPA buffer, and load 10 µl in triplicate into 96-well plate
- Add 200 µl of Reagent A & B mix per well, using a multichannel pipettor.
- Incubate at 37°C for 30 minutes with the lid on top of the 96-well plate.

- Read developed plate on a plate reader at an absorbance of 562.
- Adjust sample absorbance based on standard curve to determine concentration.