Title: Collection of frozen tissue samples for proteomic analysis.

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Purpose: The purpose of this SOP is to procure and preserve tissue samples for analysis of the labile proteome (e.g. phosphoproteins). The phosphoproteome changes rapidly (i.e., within seconds) in response to perturbation of cells, resulting in pre-analytical variables that can mask the biology of interest, and create artifacts in the data. The aim of this SOP is to minimize such preanalytical variation by flash freezing tissues (liquid nitrogen or dry ice), while avoiding (or at least minimizing) ischemia (warm and cold), exposure to anesthesia or other medications, and exposure to chemical fixatives; all of these may affect the phosphoproteome. Where it is not possible to eliminate such variables, they should be standardized and carefully recorded for each sample, such that their effects may be factored in during proteomic data analysis and interpretation.

Typical proteomic analyses of tumor tissues require an extracted protein yield of between 50 and 1,000 μg of protein (mass depends on experimental goals), with 50 μg needed for global proteome analysis and up to 1,000 μg needed for analysis of post translational modifications (PTMs) and 20 μg of co-isolated DNA and RNA. Depending on the extraction efficiency from tissue materials, it is estimated that approximately 5 to 200 mg wet tissue will be required. Protein yields are tumor-type dependent but typically rage from 1% to 5% of wet tumor mass.

Revision History

Revision date	Revision Author	Revision notes

A. Supplies

- ☐ Cryovials prelabeled with relevant information (Thermo Scientific #377224 or equivalent)
- ☐ Cryobaby labels (USA Scientific #9187-1708 or equivalent)
- ☐ Black HistoTec Pen (Newcomer Supply #6551 or equivalent)
- ☐ Dry ice
- ☐ 2 L Dewar (Fisher # 03-692-156 or equivalent), two thirds full of liquid nitrogen
- ☐ Shandon Tissue Thumb Forceps, 3 x 4 Teeth 8” (Fisher #6654 or equivalent)
- ☐ 50 mL tube with 25 mL ice-cold PBS or normal saline

B. Procedure

1. Each tissue sample should be processed using separate instruments and processing supplies to prevent cross-contamination. Processing should be as rapid as possible to minimize preanalytical variation.

2. Record:
    - Date of collection: _____
    - Time of *in vivo* blood supply cutoff of (devascularization/clamp): _____
    - Time at which the tissue is collected from the patient: _____
    - Time tissue is frozen: _____
    - Note: total time between blood supply cutoff and tissue freezing must not be greater than 30 minutes; however, preservation within 10 minutes or less is preferred. For all cases, this total ischemic time must be documented.

3. If a large tissue sample is obtained, cut the tumor into pieces of approximately 0.4 x 0.4 x 0.4 cm. If multiple pieces of tumor are submitted for analysis, they must have been collected from the same tumor nodule and as close in proximity to each other as possible.

4. If there are more than two 0.4 x 0.4 x 0.4 cm pieces of tumor, retain one piece of tumor for histology by placing it in 5 mL 4% neutral buffered formalin. (Ideally, a minimum of two 0.4 x 0.4 x 0.4 cm pieces of tumor is suggested for both proteomic analyses and confirmatory histopathology.)

5. Immediately following tissue collection, place the tissue in a 50 mL tube containing 25 mL of ice-cold PBS or normal saline to rinse away residual blood. If appropriate, gently squeeze (do not macerate) the tissue with forceps to “milk” excess blood from the tissue sample.

6. Place the tissue or tissue pieces to be frozen on top of a block of dry ice and record tissue size
    - Record information for all individual tumor pieces:

	Length (mm)	Width (mm)	Height (mm)
Tissue piece 1			
Tissue piece 2			
Tissue piece 3			
Tissue piece 4			
Tissue piece 5			
Tissue piece 6			

7. Place individual tissue samples in pre-labeled cryovials and close the vial caps.

8. Use forceps to place the tube into liquid nitrogen (liquid phase) or in dry ice until thoroughly frozen. Note the time the tubes went into the liquid nitrogen or dry ice. (The tubes should be frozen within 10 minutes of tissue excision.)

9. After collecting all tissue samples, transfer the tubes from liquid nitrogen (or dry ice) to an appropriate box and store the box either in the vapor phase of a liquid nitrogen tank or in a -80 ^{o}C freezer. Avoid freeze-thaw cycles to preserve the proteome.

10. The sample should be transported/shipped on dry ice.