ARCHIVED FFPE SECTIONS (SLIDES) FOR DISCOVERY PROTEOMIC ANALYSIS

Lab: CLIA-CAP Targeted Proteomics Laboratory, Paulovich Lab at Fred Hutch

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PURPOSE

The purpose of this procedure is to generate and ship FFPE sections (mounted on glass slides) of sufficient quality and quantity for discovery proteomics, which includes global protein abundance (TMT labeling, 24 basic reverse phase fractions).

SAMPLE REQUIREMENTS

1. There is no limit on age of blocks used for cutting sections.
2. To assure data quality, all samples should have ≥ 25% tumor cellularity, and ≤ 25% necrosis.
    a. If the sample does not meet these requirements, we will perform macrodissection if possible. Please consult with the Paulovich Lab.
3. Please record fixation time or provide a note if fixation time is not known. For all samples, fixation time in neutral buffered formalin should be a minimum of 6 hours and a maximum of 72 hours. Fixation time begins when the specimen is placed in formalin (not when sectioned during gross examination) and ends when the cassettes are no longer in formalin.
4. The required number of FFPE sections for discovery proteomics is listed below (**Table 1**).
5. We request 10 micron (µm) thick sections mounted onto glass slides for discovery proteomics.
6. In addition, we request two “bookend” sections (i.e., one section before and one section after the entire set of proteomics sections) of 4-5 µm thickness mounted onto glass slides for H&E staining. We use these slides to document tumor cellularity and when analyzing the molecular data.
    a. Staining can be done either at the sample collection site or at FHCC.
    b. Stained or unstained, bookend H&E slides should be sent to FHCC for image analysis.
7. Please label all slides with the date of sectioning and a combination of two forms of identification for tracking (e.g., a de-identified coded sample identification number, site ID, study ID, slide number).
8. All slides (bookend H&E slides plus the slides for discovery proteomics) must be vacuum packed and stored at 4°C as soon as possible (within 1 day of sectioning) to avoid preanalytical variations (e.g., oxidation).
9. Slides should be shipped for analysis as soon as possible and within 2 weeks of sectioning.

Table 1. Required Number of Sections for Discovery Proteomics

	Discovery Proteomics			Bookend H&E	
Sample	**Cross-Sectional Area**	**Section Thickness**	**Number of Sections**	**Section Thickness**	**Number of Sections**
Surgical biopsy	100 mm^2	10 µm	1	4-5 µm	2
Surgical biopsy	50 mm^2	10 µm	2	4-5 µm	2
Surgical biopsy	40 mm^2	10 µm	3	4-5 µm	2
Surgical biopsy	30 mm^2	10 µm	4	4-5 µm	2
Surgical biopsy	< 30 mm^2	Please consult with the Paulovich Lab			

INSTRUCTIONS FOR GENERATING AND SHIPPING FFPE SECTIONS

Reagents and Supplies:

- Charged slides (Leica Biosystems #3800040 or equivalent)
- Microscope slide box (Heathrow Scientific #HS15991A or equivalent)
- Silica gel packets (Electron Microscopy Sciences #71206-01 or equivalent)

Equipment:

- Microtome (Leica # RM2255 or equivalent)
- Vacuum sealer (Anova #ANVS01-US00 or equivalent)

Detailed Instructions:

1. Cut sections onto warm water bath, no more than 50°C.
2. Mount all sections on charged glass slides.
3. Label the slide numbers sequentially and annotate the thickness of the section as well.
4. Cut one bookend 4-5 µm section for H&E staining immediately before the sections for discovery proteomics analysis are collected.
    a. Staining can be done either at the sample collection site or at FHCC. Stained or unstained, this slide should be sent to FHCC for image analysis.
5. Cut the required number of 10 µm thick sections based on the sample's cross-sectional area (see **Table 1** above).
    a. 10 µm sections may be easier to cut from room temperature or lightly chilled paraffin blocks.
6. Immediately after all sections for discovery proteomics have been collected, cut one bookend 4-5 µm section for H&E staining.
    a. Staining can be done either at the sample collection site or at FHCC. Stained or unstained, this slide should be sent to FHCC for image analysis.
7. Air dry all slides overnight at room temperature.
8. Place all slides (both bookend H&E slides plus the slides for discovery proteomics) into a slide box along with a silica gel packet.
9. Vacuum pack the slide box using a vacuum sealer and store the box at 4°C.

a. All slides must be vacuum packed and stored at 4°C as soon as possible (within 1 day of sectioning) to avoid preanalytical variations (e.g., oxidation).

10. Transport or ship vacuum-packed samples on wet ice (i.e., ice packs) in a leak-proof container or outer package.
    a. Please DO NOT use dry ice.
11. Please label the outer package clearly and durably with the words “Diagnostic Specimen”, and the name, address, and phone number of both the sender and the recipient.
12. Please email the Paulovich Lab before sending samples to coordinate shipping and ensure safe receipt (preferably on a Monday; see Main Contact and Backup Contact info below).
    a. Please DO NOT ship samples on Fridays for delivery on Saturdays. If samples must be shipped for weekend delivery, please call and/or email the lab ahead of time and include the tracking number to ensure proper receipt.
13. Please ship by **Fed Ex** priority overnight or next day delivery (Fed Ex account information will be provided by email) to the following address:

    Fred Hutchinson Cancer Center
    ATTN: Rose Pletcher
    1100 Fairview Avenue North
    Paulovich Lab E2-420
    Seattle, WA 98109-1024, USA

Main Contact: Rose Pletcher
Email: rpletche@fredhutch.org
Phone: +1 (206) 667-2613

Backup Contact: Travis Lorentzen
Backup Email: tlorentz@fredhutch.org
Backup Phone: +1 (206) 667-5107

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