SOP: Sectioning and Staining of PDX Tissue for Histopathologic Assessment

Effective Date: 5/20/2021

Please check for revision status of the SOP at

https://pdmr.cancer.gov/sops/

PDMR NCI Patient-Derived Models Repository
An NCI Precision Oncology Initiative℠ Resource

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VERSION INFORMATION

1. Change History

Revision	Description
5/20/2021	New Document for PDMR Public Website

2. Related SOPs

SOP50103: Histopathological Assessment of Patient-Derived Xenografts

1.0 PURPOSE/SCOPE

Patient-derived xenograft (PDX) specimens are submitted to Mocha Histology for standard histopathology processing and evaluation from the Biological Testing Branch (BTB). Specimens are excised tumor samples and/or other murine tissues in plastic vials with screw top lids containing a minimum of 30 mL of 10% neutral buffered formalin (NBF). Following receipt specimens are processed by serial dehydration with ethanol and xylene and infiltrated with paraffin in preparation for further processing to Hematoxylin and Eosin (H&E) slides suitable whole slide scanning and pathology evaluation.

2.0 SAFETY

Lab coats, safety glasses, closed-toe shoes, and gloves must be worn when handling hazardous or sensitive equipment, samples, reagents, and materials. These safety measures must also be followed when in proximity to those who are working with these items. All activities involving 10% NBF are to be performed in a Class 2 Biological Safety Cabinet (BSC).

3.0 EQUIPMENT

3.1 Materials

3.1.1 Stainless steel forceps

3.1.2 10% Neutral Buffered Formalin (NBF)

3.1.3 70%, 95% and 100% Ethanol (ETOH)

3.1.4 Xylene, Reagent Grade

3.1.5 Paraplast

3.1.6 Lidded container

3.1.7 Biopsy cassette foam pads

3.1.8 Gauze

3.1.9 Embedding cassettes

3.1.10 Distilled water

3.1.11 +Charged glass microscope slides

3.1.12 Low profile disposable microtome blades

3.1.13 Slide staining rack

3.1.14 Ice pan

3.1.15 Indelible marker

3.1.16 Lubricating oil

3.1.17 KimWipes

3.1.18 Embedding molds

3.1.19 Tissue tampers

3.1.20 Plastic scraper

3.1.21 Hematoxylin Type 2

3.1.22 Bluing Reagent

3.1.23 Eosin Type 2

3.1.24 Micromount

3.1.25 Clarifier 1

3.1.26 Glass coverslips (24x50mm #1)

3.1.27 Flat slide folders

3.2 Equipment

3.2.1 Leica IPC Cassette Printer

3.2.2 Biological Safely Cabinet (BSC)

3.2.3 Tissue Tek VIP 6 AI Tissue Processor

3.2.4 Leica EG1150 Embedding Station

3.2.5 Premiere XH-90 wax trimmer

3.2.6 SlideMate AS

3.2.7 Leica RM2235 Manual Rotary Microtome

3.2.8 Leica AutoStainer XL-CV5030 Robotic Coverslipper Workstation

3.2.9 AT2 Whole slide Autoscanner

4.0 RECEIPT, PROCESSING, EMBEDDING, AND SECTIONING OF FORMALIN-FIXED PDX SAMPLES

4.1 Specimens are excised tumor samples and/or other murine tissues in plastic vials with screw top lids containing a minimum of 30 mL of 10% neutral buffered formalin (NBF). Prior to processing, samples are fixed for a minimum of 24 hours in 10% NBF.

4.2 Samples are assigned a Histology MoCha ID number based on the study and year. "XXXX" represents the unique sample number sequentially assigned at receipt. For example:

4.2.1 PDXCLINIC = HCL20XXXX

4.2.2 PDXDRUG = HDR20XXXX

4.3 Trimming cassettes are printed with the Specimen ID and Histology MoCha ID using the cassette printer (Leica IP C cassette writer).

4.4 Within the Biological Safety Cabinet, transfer samples from containers to the corresponding trimming cassette and place cassettes in the processing rack in a container holding 70% ETOH. Biopsy pads may be used for small samples.

4.5 Samples can be processed in batches of up to 300 cassettes in the tissue processor (Tissue Tek VIP 6 AI). Place cassette racks with lid in the processor retort and lock the retort lid. Select the standard overnight processing program and desired completion time.

4.5.1 Standard overnight processing:

Step	Solution	Time	Temperature
1	70% Ethanol	30 min	RT
2	70% Ethanol	30 min	RT
3	95% Ethanol	30 min	RT
4	95% Ethanol	30 min	RT
5	100% Ethanol	30 min	RT
6	100% Ethanol	30 min	RT
7	100% Ethanol	30 min	RT
8	100% Xylene	30 min	RT
9	100% Xylene	30 min	RT
10	100% Xylene	30 min	RT
11	Paraffin	30 min	60°C
12	Paraffin	30 min	60°C
13	Paraffin	30 min	60°C

4.6 After processing, move cassettes to the embedding center (Leica EG1150 H). Return empty cassette racks to the processor retort and run the standard clean cycle.

4.7 Place the sample tissue into an embedding mold with melted paraffin. Position tissue into the middle of the mold with forceps and move mold to the cooling block to solidify paraffin. Immediately place the cassette on top of the mold and add more paraffin to fill cassette. Place mold/cassette on the cold plate to solidify (Leica EG1150 C).

4.8 Once cassettes are solidified, remove the mold and scrape excess paraffin from the block with the wax trimmer (Premiere XH-90).

4.9 PDX FFPE blocks are next sectioned using the Leica RM2235 Rotary Microtome from Leica Biosystems.

4.9.1 Fill the water bath with distilled water and equilibrate to 45°C for at least 30 minutes prior to sectioning.

4.9.2 Temperature may be adjusted as necessary during sectioning to facilitate tissue ribbon quality and adherence to the slide.

4.9.3 Face each block, taking care to conserve as much tissue as possible.

4.9.4 Soak all blocks on ice tray before sectioning.

4.9.5 Slides can be labeled by hand or with a slide printer.

4.9.6 Blocks are sectioned at 5 *u*m thickness. Ensure that the sections are mounted to the corresponding slide(s).

4.9.7 Sectioned slides are left to dry overnight at room temperature and may remain at room temperature until H&E staining.

5.0 ROUTINE H&E STAINING AND COVERSLIPPING FOR PDX SAMPLES

5.1 All PDX samples are stained with hematoxylin and eosin using Leica Biosystem's Autostainer XL (ST5010) and automatically cover-slipped with the Robotic Coverslipper (CV5030). The Autostainer XL stains up to 30 slides in a single rack which may be continuously loaded until all slides are stained. Stainer can stain 200 slides/hour.Set up Robotic Coverslipper prior to staining

5.1.1 Autostainer XL Set up and Staining

5.1.1.1 Control Slide is run to check quality of the H&E stain prior to loading any PDX slides.

5.1.1.2 Load PDX slides one rack at a time. Stained slides will automatically transfer to the coverslipper.

5.1.1.3 Remove slides individually and check for bubbles, incomplete mounting media, broken coverslip, etc. and recover-slip as necessary. Place slides in a slide flat to dry overnight.

Program 2: FFPE

Station	Reagent	Time (min:sec)
Oven	---	10:00
1	100% Xylene	4:00
2	100% Xylene	4:00
3	100% Xylene	4:00
4	100% ETOH	4:00
5	100% ETOH	4:00
6	95% ETOH	1:30
Wash 1	Tap water	1:00
8	Hematoxylin	3:00
Wash 5	Tap Water	1:00
9	Clarifier	1:00
Wash 4	Tap Water	1:00
10	Bluing	0:20
Wash 3	Tap Water	1:00
11	95% ETOH	1:00
12	Eosin	0:20
13	95% ETOH	3:00
14	100% ETOH	3:00
15	100% ETOH	3:00
16	100% ETOH	3:00
17	100 % Xylene	3:00
18	100 % Xylene	3:00
Exit	100 % Xylene	HOLD for coverslipping

6.0 QUALITY CONTROL AND WHOLE SLIDE SCANNING OF PDX

6.1 Ensure the number of blocks and slides match the number of specimens on paperwork.

6.2 Slide block match each specimen to ensure correct sample mounted on the correct slide.

6.3 Review all H&E slides under a light microscope.

6.3.1 Check section and staining quality and recut if any significant artifacts noted. Artifacts include, but are not limited to: dryness, rough cut holes, incomplete section, uneven staining, etc.

6.4 Print barcode labels from the appropriate barcode manifest. Double check labels to ensure information matches paperwork and affix barcode labels to slides.

6.5 Load clean slides on the scanner. Slides will scan to a local server.

6.6 Review image for quality control; examples include but not limited to debris, blurriness, cut off, etc.

6.7 File slides and paperwork in appropriate location.