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Version 2

Sinai SCENT TMC - FFPE Blocking, Sectioning, and TMA Construction V.2

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Sojin Kim¹

¹Icahn School of Medicine at Mount Sinai

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Sojin Kim

Icahn School of Medicine at Mount Sinai

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Protocol status: Working

We use this protocol and it's working

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Abstract

FFPE Blocking and Sectioning protocol

Guidelines

Comply with Universal Precautions when handling all specimens.

Use personal protective equipment according to the institution's guidelines.

Safety warnings

❗ Do not allow tissue samples to dry at all (One-at-a-time).

Before start

Tissue collection for this protocol needs prior approval by the users' Institutional Animal Care and Use Committee (IACUC) or equivalent ethics committee.

FFPE Blocking and Sectioning

- 1 Rinse the collected tissues in PBS to remove blood.
- 2 Place tissues in at least 10 volumes of buffered formalin or buffered paraformaldehyde
- 3 Incubate for the necessary fixation time
 1. 1-2 mm thick: 2-3 hours RT
 2. 5-10 mm thick: 5 hours RT
 3. >10 mm thick: 2-3 hours RT with overnight at 4°C
- 4 Rinse tissue twice with PBS, and store at 4°C in 70% EtOH
- 5 Label sample ID on both the front and side of the histology cassettes
- 6 Trim and transfer the tissue in the histology cassettes. Small tissue pieces < 1-2mm should be placed between blue sponges within the cassette.
- 7 Immerse in the 70% EtOH in the storage bucket
- 8 Drop off the samples at the Biorepository and Pathology CoRE (Mount Sinai)
- 9 Sectioning thickness
 - Xenium: 5 µm
 - Orion: 5 µm

H&E

- 10 H&E staining (5 µm) is performed for general histopathological analysis and TMA construction.

TMA Construction

- 11 Donor blocks are selected by the researcher and pathologist based on study criteria.



Exclusive criteria

1. Artifacts such as folding or tearing
2. Areas with excessive bleeding

Inclusive criteria (for Lung tissues)

Alveolar, bronchial, and endothelial, while trying to select the same anatomical regions among the samples.

- 12 The region of interests are marked using the NDP.view 2 program and submit the annotated H&E scan to the Biorepository and Pathology CoRE Team at Mount Sinai.