

Jul 31, 2024

Version 1

Sinai SCENT TMC - FFPE Blocking, Sectioning, and TMA Construction V.1

DOI

<https://dx.doi.org/10.17504/protocols.io.5jyl82wjrl2w/v1>

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Protocol Citation: Sojin Kim 2024. Sinai SCENT TMC - FFPE Blocking, Sectioning, and TMA Construction. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.5jyl82wjrl2w/v1>

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Protocol status: Working

We use this protocol and it's working

Created: July 31, 2024



Last Modified: July 31, 2024

Protocol Integer ID: 104376

Keywords: tma construction ffpe blocking, ffpe blocking, sinai scent tmc, sectioning, protocol, sinai scent

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)

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

TMA Construction

- 10 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.

- 11 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.