Cryopreservation

Michael Eadon[1], Daria Barwinska[1], Ying-Hua Cheng[1], Michael J. Ferkowicz[1], Samir V. Parikh[2], Brad H. Rovin[2], John P. Shapiro[2], Pierre C. Dagher[1], Tarek M. El-Achkar[1]

[1]Indiana University School of Medicine, [2]Ohio State University Wexner Medical Center

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1 Works for me

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KPMP
Tech. support email: info@kpmp.org

Brandi Gizinski

ABSTRACT

Samples of kidney tissues are cryopreserved, sectioned and processed for various analyses. KPMP biopsy tissue undergoes initial processing (e.g. OCT embedding) at the Recruitment Sites, prior to distribution to Tissue Interrogation Sites. KPMP pilot nephrectomies were cryopreserved at University of Michigan. IU biopsies and nephrectomies were cryopreserved as described below. The tissue is sectioned according to a predetermined order: Bulk, LMD, miRNA, proteomics at 12 µm and then 50 µm sections for imaging studies as described in Section 4.4. This allows efficient use of the edge tissue and maximization of imaging data that will anchor the LMD information with the remainder of the biopsy to be sent to other Tissue Interrogation Sites for further interrogation. Shared tissue blocks received from other Tissue Interrogation Sites will be sectioned in the reverse order.

GUIDELINES

This protocol is to be used for 0.5-10 mm^3 pieces of kidney tissue, including local nephrectomies and biopsies.

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1

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Kidney tissues are acclimated to OCT for **00:03:00** and then transferred to a cryomold with partially frozen OCT in the bottom (on a block of dry ice).

2 The tissue is completely covered in OCT, avoiding bubbles.

3 When the OCT is completely frozen, the tissue block is wrapped in parafilm (aiming to expel all air), then placed in a sealed bag and stored at **-80 °C**

Cryosectioning Procedure

4

This protocol is to be sued with kidney tissue preserved in OCT and store at **-80 °C**

All work is performed in a manner that limits RNA contamination and necessitates use of clean disposable gloves and face mask, as well as ensuring the cleanliness of all surfaces (RNAse Away, Ambion, Cat #10328011).

5 1.2 µm Leica PPS-membrane slides (Leica, Cat# 11505268) used for LMD are exposed to UV light (in a tissue culture laminar flow hood) for 30 minutes, immediately prior to cryosectioning. Slides are stored at room-temp for optimal tissue adherence.

6 Cryostat is cooled to **-22 °C** . The work surfaces are cleaned and a new cutting blade is installed.

7 A small slide box (cleaned with RNAse Away) is placed inside the cryostat chamber to store slides with freshly cut tissue.

8 Specimen in OCT is adhered to a tissue holder and allowed to equilibrate for a few minutes to reach the chamber temperature and strengthen the adhesion between the OCT block and the holder. This process is aided via use of a heat extractor.

9 The specimen is cut at the following thickness:
a) 12 µm (2 section) – placed in Eppendorf tube for Bulk analysis
b) 12 µm (8 sections) – affixed to the specialized Leica LMD slide; one nephrectomy section per slide or two biopsy sections per slide. The slides are stored in **-80 °C** , with a desiccant cartridge (Bel-Art, Cat# F42046-0000). To prevent any moisture from accumulating inside the slide box, it is further stored in a tightly closed Ziplock® bag.
c) 12 µm (1 section) – affixed to a glass slide for Periodic acid-Shiff staining
d) 12 µm (2 sections) – miRNA
e) 12 µm (6 sections) – For proteomics, tissue is affixed to specialized PEN-membrane LMD slides; one nephrectomy section per slide or two biopsy sections per slide. The slides are stored in **-80 °C** , with a desiccant cartridge prior to shipment to OSU.
f) 50 µm (2 sections) – stored in tissue culture plate with 4% PFA for 24hr, followed by transfer of tissue into a scintillation vial with 0.25% PFA for long term storage. The tissue in 0.25% PFA is stored at 4°Celsius at all times when not in use. All sections per donor can be placed into the same storage container. Subsequent imaging of 50 um slices is described in section 4.4

10 The LMD membrane slide adapter is used to assist with tissue adherence and collection.

11 Each slide is labeled with a specimen ID, date, and slide number.

12 Slides are used within 10 days from the date of cryosectioning.

13 For specimens transferred elsewhere for processing, the tissue is shipped (Mon-Thurs) overnight on dry ice to be received at the destination facility the following business day.