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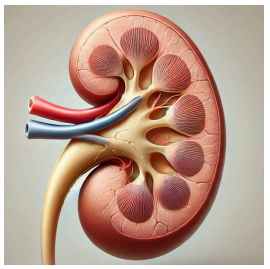
Preparation of Mouse Kidney for Spatial and Single-Cell Transcriptomics



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We use this protocol and it's working

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Abstract

This protocol describes the preparation of murine kidney specimens for single-cell and spatial transcriptomics through a non-survival procedure. The protocol can also be adapted for immunohistochemistry, immunofluorescence, or other molecular assays.

Image Attribution

Image generated by OpenAI's GPT-4o model

Materials

10cm dish can be used instead, too.

- Corning® non-treated culture dishes 60 mm × 15 mm **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**CLS430589

Eppendorf Tubes 5.0 mL, screw cap, (Catalog No. 0030122313)

Integra Miltex Sterile Disposable Scalpels, Blade No. 10 (Fisher, Catalog No. 12-460-451)

Protocol materials

- Ethyl alcohol, Pure 200 proof, for molecular biology **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**E7023
- Paraformaldehyde Aqueous Solution -16% **Electron Microscopy Sciences Catalog #**15700
- Gibco™ DPBS no calcium no magnesium **Thermo Fisher Scientific Catalog #**14190144
- Dulbeccos phosphate-buffered saline (DPBS) **Gibco - Thermo Fisher Scientific Catalog #**14190144
- 16% Paraformaldehyde **Electron Microscopy Sciences Catalog #**15710
- PBS - Phosphate-Buffered Saline (10X) pH 7.4 **Thermo Fisher Scientific Catalog #**AM9625
- Corning® non-treated culture dishes 60 mm × 15 mm **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**CLS430589

Safety warnings

This protocol involves animal surgical procedures that must be conducted in strict accordance with all applicable laws, regulations, and ethical standards. Failure to adhere to these guidelines may result in legal consequences, including revocation of research privileges, sanctions, or other enforcement actions.

Ethics statement

The animal handling procedures described in this protocol are provided solely as an illustrative example and must not be implemented without proper approval. The sole authoritative source for the execution of animal handling procedures is the protocol approved by the local Institutional Animal Care and Use Committee (IACUC), in strict accordance with applicable laws, regulations, and institutional policies designed to protect animal welfare.

Following this described method is not a substitute for exercising the utmost diligence and care required when handling animals. Researchers are advised to prioritize animal welfare and scientific integrity at every stage of their work. Caution should be exercised to ensure compliance with all relevant ethical and legal standards, and any deviation from approved protocols must be justified and formally authorized by appropriate regulatory bodies.

By proceeding with this protocol, you acknowledge and agree to adhere to all applicable legal and ethical requirements and to accept responsibility for ensuring the humane and lawful treatment of animals throughout the course of these procedures.

Before start

Prepare all required sterile surgical instruments, including scissors, forceps, and a scalpel.
Razor blades may be used as an alternative to a scalpel, provided they are sterilized.

Precool Dulbeccos phosphate-buffered saline (DPBS) **Gibco - Thermo Fisher Scientific Catalog #**14190144

For FFPE fixation, dilute EM-grade
16% Paraformaldehyde **Electron Microscopy Sciences Catalog #**15710 from a new ampoule in
PBS - Phosphate-Buffered Saline (10X) pH 7.4 **Thermo Fisher Scientific Catalog #**AM9625 to make fresh 4% PFA solution shortly before the process.

Animal handling as non-survival surgical procedures

1 Euthanize the mouse using an approved method to ensure minimal distress, adhering to existing IACUC protocols.

A surgical plane of anesthesia must be established before starting the procedure and consistently maintained throughout the surgery until euthanasia is performed.

Safety information

Warning: The procedures outlined in this protocol are examples and should not be applied without approval from your local IACUC and adherence to relevant legal and institutional regulations protecting animal welfare. The exact method used must align with the specific disease model and biological questions being addressed. Improper application may compromise animal welfare and confound experimental outcomes. Maximal caution and due diligence are essential to ensure scientific integrity and ethical compliance.

1.1 *[Example]* Anesthetize mice using
1. CO_2 asphyxiation (per AVMA guidelines with appropriate flow rates) or
2. inhalation of <5% isoflurane in a properly ventilated hood.

Confirm successful induction of anesthesia before proceeding by checking for the absence of reflexes (e.g., toe pinch test).

1.2 *[Example]* Apply confirmatory cervical dislocation. Secure the head with one hand and the hindquarters or tail with the other. Perform a swift and firm motion to separate the cervical vertebrae.

Confirm death by checking for absence of reflexes and respiration.

1.3 Immediately proceed to the tissue collection steps.

Harvesting and grossing

2 Place the mouse in the supine position on a sterile surgical pad.

3 Using sterile dissecting scissors, create a midline incision along the linea alba, starting from the pubis and extending cranially toward the xiphoid process.

4 Reflect the skin laterally to expose the abdominal wall (musculature and fascia).

5 Incise the abdominal musculature and peritoneum along the same midline axis, ensuring clean entry into the peritoneal cavity. Avoid damaging underlying viscera.

6 Gently retract the intestines laterally using sterile forceps or moistened gauze to expose the kidneys in their retroperitoneal location.

7 Identify the renal fascia and dissect it away using fine forceps or iris scissors to isolate the kidney.

8 Excise each kidney promptly to minimize warm ischemic time (WIT) by transecting the renal pedicle, including the renal artery, renal vein, and ureter using microsurgical scissors.

9 Gently recover the kidney individually, minimizing mechanical damage to the renal capsule.
Immediately immerse the kidney in an appropriate medium, such as RNAse-free ice-cold phosphate-buffered saline (dPBS).

Gibco™ DPBS no calcium no magnesium **Thermo Fisher Scientific Catalog #**14190144

10 Using fine forceps, carefully dissect and remove the perinephric fat in a tissue culture dish.

11 Using fine surgical scissors, excise a portion of the renal pelvis at its base to eliminate remnants of the ureter.

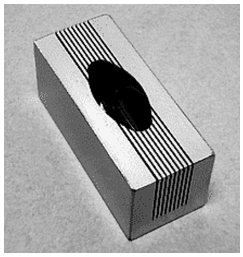
12 Superficially pierce the fibrous renal capsule using a fine needle or scalpel blade.

13 Grasp the capsule at the opening with fine forceps and gently peel it away from the kidney surface, taking care to avoid damaging the underlying renal parenchyma.

14 Bisect each kidney along the sagittal plane using a sterile, sharp scalpel to ensure clean and precise cuts.

Note

Alternatively, consider using a commercial stainless block or 3D-printed resin block.



Example of a matrix block with a 0.5 mm channel

15 Keep one half for FFPE and the other half for FF. Or further divide into quadrants for different types of assays and preservation.

16 Rinse the sample again in RNase-free

Gibco™ DPBS no calcium no magnesium **Thermo Fisher Scientific Catalog #**14190144

to remove blood.

17 Immediately proceed to fixation and freezing.

Fixation, processing and embedding (FFPE-option)

3d 2h 15m

18 Fix the kidney halves in fresh methanol-free EM-grade [M] 4 % (v/v) paraformaldehyde (PFA) at 4 °C Overnight in a RNase-free screw cap 5 mL tube, ensuring the tissue is fully submerged. Gently shake on an orbital shaker.

Note

Always prepare fresh PFA from methanol free EM-grade PFA out of a new ampoule.

Paraformaldehyde Aqueous Solution -16% **Electron Microscopy Sciences Catalog #**15700

Unused PFA can be stored shaded from light at 4 °C for no more than 72:00:00 .

19 In the next morning, remove the fixative and replenish the tube with fresh [M] 4 % (v/v) paraformaldehyde (PFA).

20 If histologist cannot immediately process the sample, dehydrate the samples in an ascending ethanol series:

Ethyl alcohol, Pure 200 proof, for molecular biology **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**E7023

20.1 [M] 70 % (v/v) ethanol 00:15:00 15m

20.2 [M] 90 % (v/v) ethanol 00:15:00 15m

20.3 [M] 100 % (v/v) ethanol 00:15:00 15m

20.4 [M] 100 % (v/v) ethanol 00:15:00 15m

20.5 [M] 100 % (v/v) ethanol 00:30:00 30m

20.6 [M] 100 % (v/v) ethanol 00:45:00 45m

20.7 store them in [M] 100 % (v/v) ethanol before submission to histology for further processing and embedding. The storage time should be limited to less than 72:00:00 . 3d