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Preparation, Processing and Preservation of Deceased Donor Kidney Tissue for Multiomic Studies

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We use this protocol and it's working

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Abstract

Multiomic technologies are increasingly being used on human samples and generating multidimensional data. Preanalytical and tissue procurement factors can have a lasting and negative effect on omic results. For these data to be biologically and clinically useful, it is essential that high quality samples are obtained with rigor and reproducibility. By using kidney tissue procurement as an example, we present an end-to-end detailed pipeline from deceased donor patients from consent to tissue procurement, processing and preservation. This approach has been extensively tested on various atlasi projects and multiple tissue types including the Human Biomolecular Atlas Program (HuBMAP) and the Kidney Precision Medicine Project (KPMP).

Materials

Refer to the main protocol for all materials
Dry Ice
Wet Ice
LN2
LN2 Transport Container
Styrofoam Container for LN2
Labels
Sharpie
Scalpel with blades
Tweezers
Forceps, long and short
Tissue Tek #4791 short blade scalpel handle with blades
Tissue Tek O.C.T. Compound
Tissue Tek Cryomolds, standard and intermediate size
Ambion RNAlater
PBS
Electron Microscopy Science Paraformaldehyde 16% solution
2cc and 5cc cryovials
Document for specimen collection, i.e., type of specimen, location (upper/lower/mid pole), processing medium, time processed, etc.

Troubleshooting

Safety warnings

Take necessary safeguards and protective ware for handling biohazardous materials and working with liquid nitrogen

Before start

Please review the entire protocol for necessary preparation and items needed including lines of communications before starting

Procurement: Procuring Kidney from organ procurement organization (OPO)

1 Check patient file for the following information:
- Consent to donate organs
- Downtime, if any
- Was kidney on pump?
- BUN/Cr
- Kidney biopsy report/any underlying disease
- Medical history for comorbidities
- General good health before the current event
- Cold ischemia time

Note

Tip: For reference kidneys exclude history of CKD, congenital anomalies, kidney infection, cold ischemia greater than 30h and age inappropriate histopathologic abnormalities

2 Arrange time of boxed organ pickup.

3 Upon arrival to OPO:

3.1 Confirm case number matches package labeling.

3.2 Confirm documentation is packaged with organ.

3.3 Document **Left OPO time** on *Worksheet* (find copy of *Worksheet* below):

4 Transport kidney to lab for processing.

4.1 Document **Arrived in Lab time** on Worksheet.

Preparation: Before Kidney Arrival

5 Prepare Worksheet with initial information (find copy of Worksheet below):

- *PPID*
- *Date* (of collection)
- *Laterality*
- *Date/Time of Extubation*
- *Date/Time of Perfusion*

PPID:	Date/Time of Extubation:	Left OPO: Arrived in Lab:	Kidney (cm):
	Date/Time of Profusion:	Total Transport Time (min):	Ureter (cm):
Date:	Warm Ischemic Time (min):	Processing Start Time:	Fixation Stop Date/Time:
Procedure: Deceased Donor Kidney	Cold Ischemic Time (min):	Processing Stop Time:	Total Fixation Time (min):
Laterality: ☐Right ☐Left	Total Ischemic Time (min):	Total Processing Time (min):	Processor's Initials:

- Specimen Labels (i.e., "K23-00001" - We use "K" for KTRC + the year followed by the next consecutive specimen number. Each new year begins at 00001, and so on.)

Specimen Label	K23000	K23000	K23000	K23000	K23000	K23000	K23000	K23000
Specimen Location	Distal Ureter Start Time: Stop Time:	Proximal Ureter Start Time: Stop Time:	Region A ↑ ↓ mid ant post Start Time: End Time:	Region B ↑ ↓ mid ant post Start Time: End Time:	Region C ↑ ↓ mid ant post Start Time: End Time:	Region D ↑ ↓ mid ant post Start Time: End Time:	Pelvis Start Time: End Time:	Papilla Start Time: End Time:

6 Label preliminary collection cassettes and tubes using the unique Specimen Labels prepared on the *Worksheet*. The table below indicates an estimate of the number of prelabeled cassettes and tubes that may be needed at the time of collection. (Have extra available for additional desired tissue).

	A	B	C	D	E	F	G	H
	Estimate of the number of cassettes and tubes to label ahead of time for each section:	**Distal Ureter**	**Proximal Ureter**	**Region A**	**Region B**	**Region C**	**Pelvis**	**Papilla /Calyx**
	Smaller Cassettes - Tissue-Tek Cryomold Intermediate size (#4566); (for O.C.T. frozen blocks – used for distal ureter, proximal ureter, cortex and other smaller pieces of tissue)	3	3	4	4	4	1	1

	A	B	C	D	E	F	G	H
	Larger Cassettes - Tissue-Tek Cryomold Standard size (#4557); (for O.C.T. frozen blocks – used for cortex/medulla pieces and other larger pieces of tissue)			4	4	4		
	**2mL vials (for 4% PFA samples, pre-fill ¾ of the way with 4% PFA – more than one section of tissue can go in tube - will later be separated and used for fixed frozen blocks (FFB) and/or paraffin blocks (PB))	1	1				1	1
	**5mL tubes (for larger tissue pieces) (for 4% PFA samples, pre-fill ¾ of the way with 4% PFA – more than one section of tissue can go in tube - will later be separated and used for fixed frozen blocks (FFB) and paraffin blocks (PB))			1	1	1		
	2mL cryovial (for Fresh Frozen LN2 - no fluid in tubes)	1	1	1	1	1	1	1
	1.5mL tubes (for RNA samples, pre-fill ¾ of the way with RNALater)	1	1	1	1	1	1	1
	Optional 5mL tubes (for additional fresh samples, fill tube ¾ of the way with PBS)			1	1	1		

**The specimens stored in the 4% PFA tubes will later be separated into tubes with sucrose for fixed frozen blocks (FFB) or EtOH for paraffin blocks (PB) – (Reference Processing Instructions for 4% PFA Samples for instructions)

7 Prefill the pre-labeled tubes (3/4 full) with 4%PFA, PBS or RNA for faster processing once kidney arrives

7.1 Reference *Supply List_Processing Kidney Tissue* Section (D) *Processing Liquids* for information on liquids needed and how to prepare solutions.

7.2 Immediately place prelabeled 4% PFA and PBS containers in wet ice.

8 Prepare processing area

8.1 Clean work area, prepare as if working in sterile conditions

8.2 Treat any area where RNA samples will be processed with RNAse Zap to prevent RNA degradation

9 Gather Supplies:

9.1 Prepared **Wet Ice** container - include:
i. Pre-labeled 4% PFA and PBS tubes
ii. Additional PBS
iii. Additional 4% PFA
iv. Glass Petri dish(es) (Petri dishes should be chilled to keep dissected tissue section cold before processing and preservation)

9.2 Prepared **Dry Ice** container (break up/powder dry ice – aides in closer contact with sample cassette and keeping it frozen)

Note

Tip: If freezing on dry ice, placing cassettes on powdered dry ice minimizes freezing artifacts due to slow freezing.

9.3 **Small styrofoam cooler** with **embedding well** (or metal block) placed inside for freezing

9.4 **LN2 transport container** (do not fill until kidney is ready)

9.5 Additional (**unlabeled**) **cassettes** and **tubes** for additional tissue (*Reference Supply List* section *(E) Processing Tissue Containers*)

9.6 Additional **RNA** & **RNAse Zap** for additional RNA collections

9.7 **O.C.T**

9.8 Remaining autoclaved items - **scalpel, long forceps**, and **regular forceps** (*Reference Supply List section (B) Autoclaved Supplies*)

9.9 Processing Tools – **clipboard** with **Worksheet_Deceased Donor Kidney Specimens, pencil, Sharpie, scalpel blades, cutting board, (2) 4" x 4" squares of parafilm sheets, ruler,** and **camera** (*Reference Supply List section (C) Processing Tools*)

Processing: Preparing Kidney

10 Fill **LN2 Transport Container.**

11 Prep kidney for dissection:

11.1 Prepare cutting board to receive kidney.

11.2 Trim surrounding fat and large vessels to expose kidney and ureter.

11.3 Orient the kidney and attached ureter on cutting board.

11.4 Identify posterior and anterior aspects.

11.5 Identify upper, mid, and lower poles.

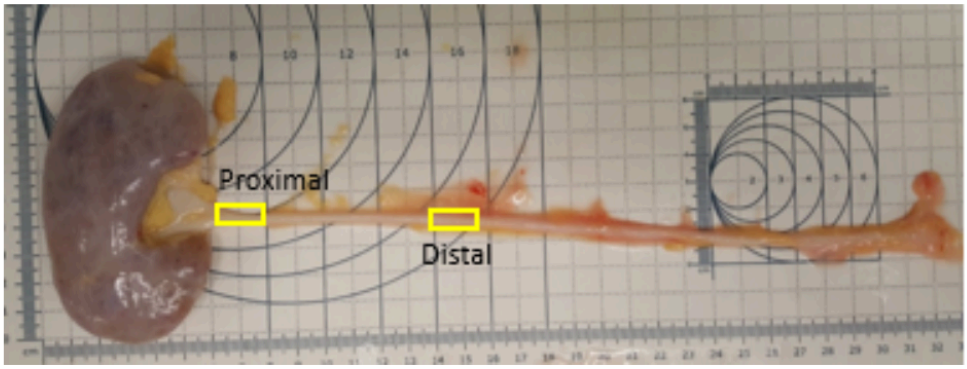
11.6 Identify pelvis.

11.7 In centimeters measure and document on *Worksheet*:
(i) *Kidney (cm)* - (L x W x D)
(ii) *Ureter (cm)* - from ureteropelvic junction (UPJ) to distal end

11.8 Take pictures of entire specimen - including ureter (place ruler in background if no printed measurements on cutting board)

Note

Tip: Pictures will be used to determine locations from which tissue was taken and/or distance from a specific reference point (i.e., superior and/or inferior pole(s) or the hilum). This will aid in placing tissue blocks in a common coordinate system.



11.9 Pour LN2 in cooler up to the top of metal embedding well (or block). Do not cover top surface of block. LN2 may need to be replenished several times while processing.

Processing: Kidney Dissection

12 Distal Ureter:

12.1 Document *Start Time* for Distal Ureter and overall *Processing Start Time* on *Worksheet*.

12.2 Obtain approximately 1-3cm of distal ureter.

12.3 Cut into roughly 2mm cross sections and allocate for desired processing.

12.4 Process Distal Ureter segments following **Processing Instructions for different preservation methods** found below.

12.5 Document *Stop Time* and sample types collected for distal ureter.

13 Proximal Ureter:

13.1 Document *Start Time.*

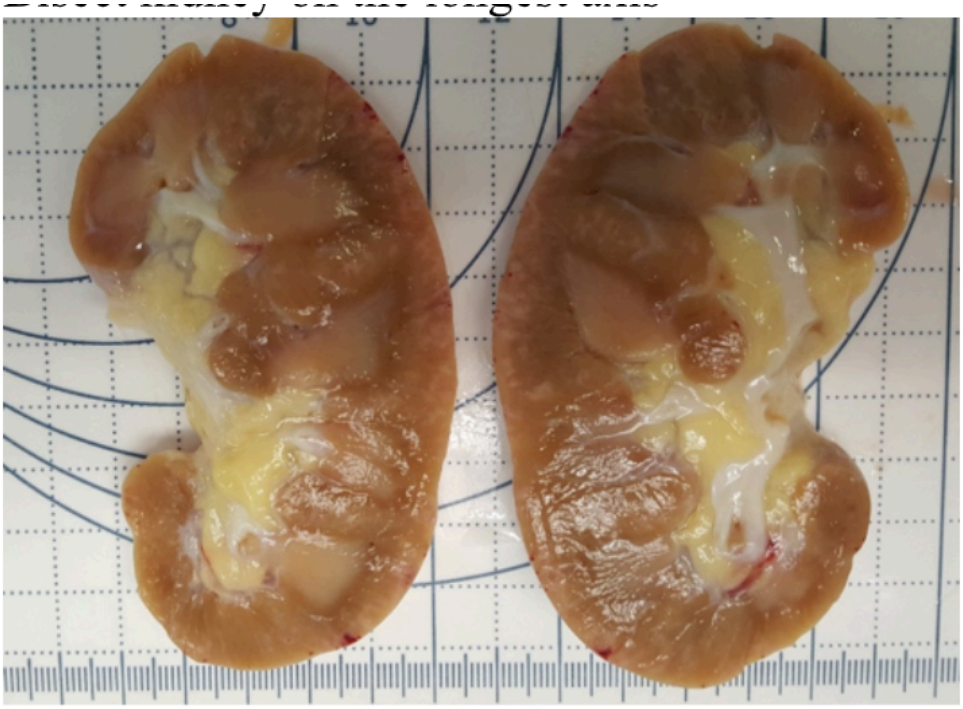
13.2 Obtain approximately 1-3cm of proximal ureter (*proximal ureter boundary is at UPJ*).

13.3 Cut into roughly 2mm cross sections and allocate for desired processing.

13.4 Process Proximal Ureter segments following ***Processing Instructions*** **for different preservation methods** found below.

13.5 Document *Stop Time* and sample types collected for proximal ureter.

14 Bisect kidney on the longest axis.

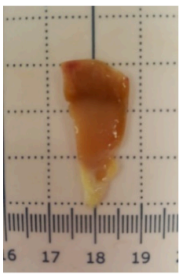
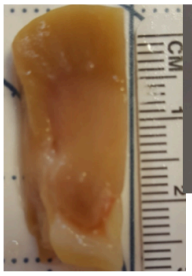


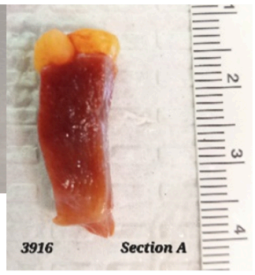
14.1 Take picture of open kidney (*include ruler if not on cutting board*).

14.2 Examine kidney for any gross abnormalities and document.

14.3 Select healthy appearing lobe(s) for dissection (the lobe typically includes an outer cortex and underlying medullary pyramid).

14.4 Carefully cut out first selected lobe containing cortex and medulla, and assign as Region A (“Region A” is shown as “Section A” in examples below).

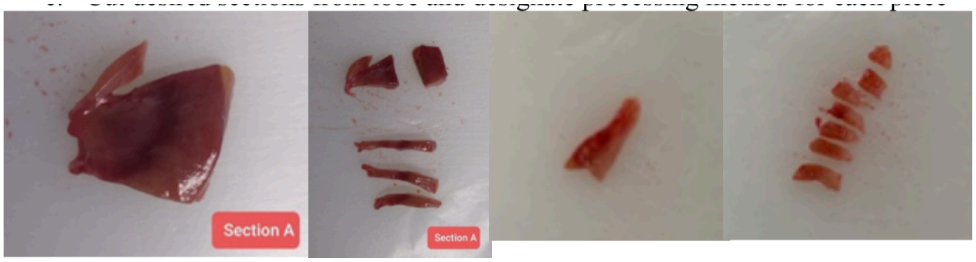



15 Region A:

15.1 Document *Start Time*, pole location, and anterior or posterior.

15.2 Take a picture of Region A (*include ruler*).

15.3 Cut desired sections from lobe and designate processing method for each piece.



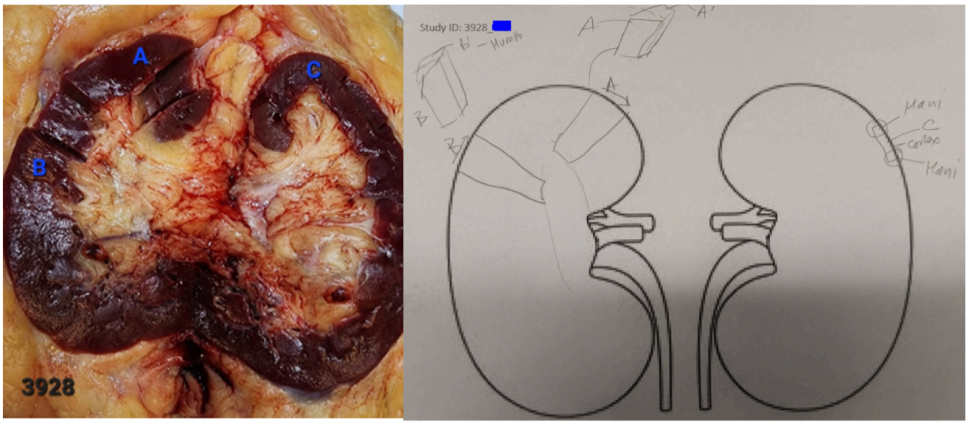
15.4 Process Region A pieces following **Processing Instructions for different preservation methods** found below.

15.5 Document *Stop Time* and sample types collected for Region A.

16 Repeat process for Regions B, C ...

17 If possible, after all lobes have been collected, take an additional picture of bisected kidney to aid in identifying the locations from where the tissue was dissected (see picture below).

18 On kidney drawing, document location of collected lobes as a reference for cut sections shown in picture.



Processing: Final Documentation

19 Document *Processing Stop Time.*

20 Confirm that each sample is accounted for and correctly marked on *Worksheet.*

21 Calculate & document *Total Transport Time* in minutes (Elapsed time from *Left OPO* time to *Arrived in Lab* time).

22 Calculate & document *Warm Ischemic Time* in minutes (Elapsed time from *Date/Time of Extubation* to *Date/Time of Perfusion*).

23 Calculate & document *Cold Ischemic Time* in minutes (Elapsed time from *Date/Time of Perfusion* to *Processing Start Time*).

24 Calculate & document *Total Ischemic Time* in minutes (Total of Warm Ischemic Time and Cold Ischemic Time).

25 Calculate and document *Total Processing Time* in minutes (Elapsed time from *Processing Start Time* to *Processing Stop Time*).

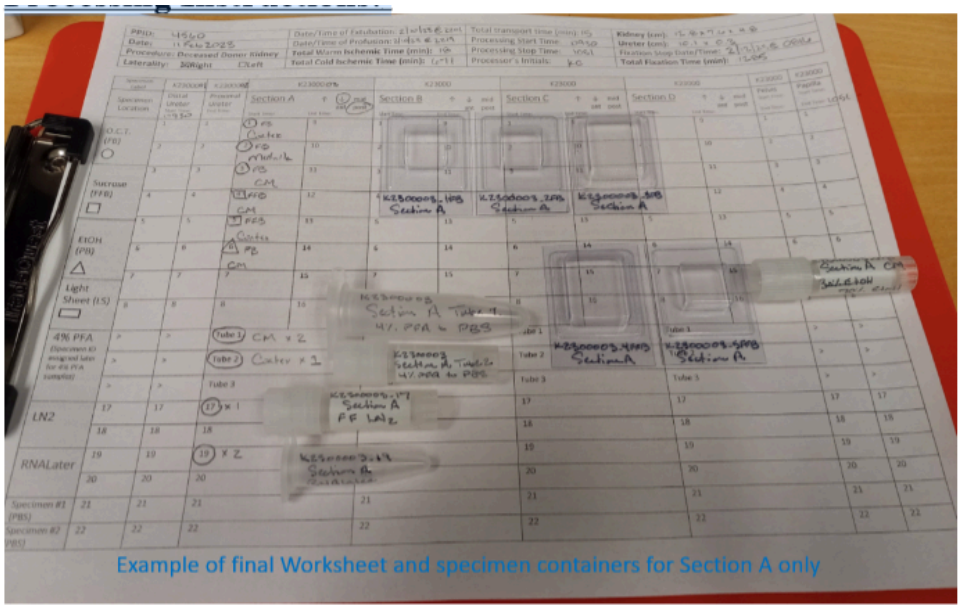
26 Document *Processor's* Initials

27 The following day, when the 4% PFA is changed to PBS, document the *Fixation Stop Date/Time* on *Worksheet.*

28 Calculate and document Total Fixation Time in minutes (Elapsed Time from *Processing Start Time* to *Fixation Stop Date/Time*).

Processing Instructions: Different Preservation Methods

29



Example of final Worksheet and specimen containers for Section A only

Fresh Frozen Tissue (FB): OCT embedding

30 Squirt a quarter sized amount of O.C.T on a 4″ x 4″ square of paraffin film.

31 Using regular forceps, gently roll cut tissue selection in O.C.T. so it is completely covered.

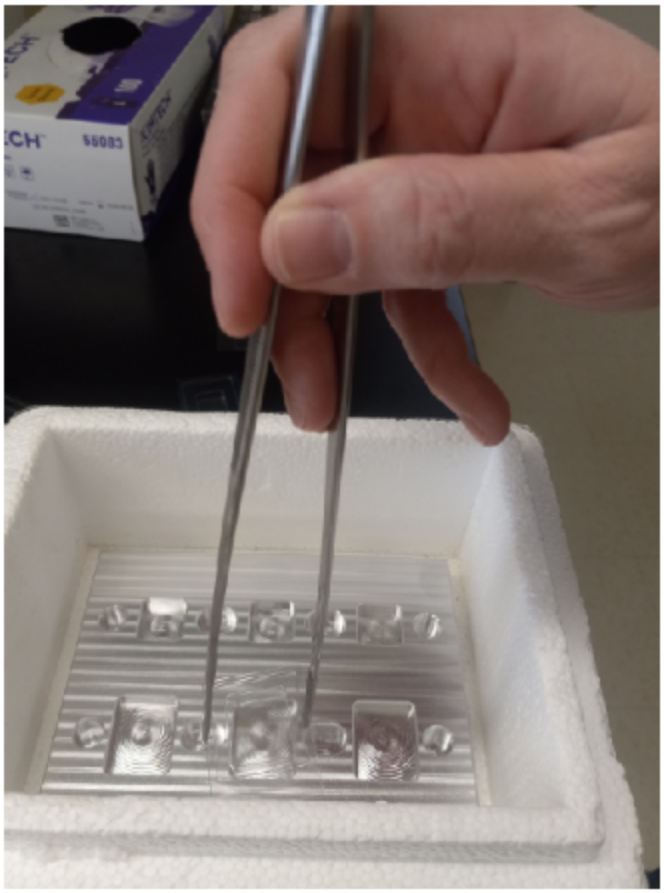
32 Place O.C.T. covered tissue strategically in labeled cassette.

33 Squirt additional O.C.T. into cassette well until tissue is completely covered.

34 With large forceps, carefully place filled cassette onto embedding well or metal block within the LN2 cooler.
NOTE: The metal block or plate should be prechilled in LN2. After pacing the metal block in the cooler or styrofoam container, fill the container with LN2 to upto half the depth of the metal block. Initially the LN2 will bubble but then will settle. Replenish if the LN2 evaporates to keep the block chilled. This usually takes about 5 -10 min. Wear protective gear to protect yourself from accidental LN2 splashes or spills.

35 Cover cooler and monitor carefully.

36 Once block is completely frozen, use large forceps to remove frozen cassette from cooler and place on bed of powdered dry ice.



Carefully place the tissue embedded in OCT cryomold into the prechilled metal block. Maintain the LN2 level below the top of the metal plate.

37 Document sample type and number – *using our nomenclature, circle "1" under correct tissue column to indicate unique specimen label (i.e., K2300003_1FB - This is your first piece of fresh frozen tissue collected. Your second piece of fresh frozen tissue under this parent number would be K2300003_2FB); see example Worksheet at the beginning of this section.*

38 When finished processing place in labeled resealable bag for protection.

39 Sample is ready for -80 °C storage.

Flash Fresh Frozen LN2 Samples:

40 Using regular forceps, gently place cut tissue selection in empty labeled tube and place lid.

41 With large forceps place tube directly in LN2 within the cooler.

42 Cover cooler and monitor carefully.

43 Once completely frozen, use large forceps to remove tube from cooler and place on bed of powdered dry ice.

44 Document sample type and number – *using our nomenclature, circle "17" under correct tissue column to indicate unique specimen label (i.e., K2300003_17 - This is your first piece of flash frozen tissue collected. Your second piece of flash frozen tissue under this parent number would be K2300003_18); see example Worksheet at the beginning of this section.*

45 Sample is ready for -80 °C storage.

Preservation in RNAlater for RNA:

8h

46 Using regular forceps, gently place cut tissue selection in labeled tube (filled ¾ of the way with RNAlater).

47 Place lid and temporarily store in Wet Ice container until all processing is complete.

48 Document sample type and number – *using our nomenclature, circle "19" under correct tissue column to indicate unique specimen label (i.e., K2300003_19 - This is your first piece of RNA treated tissue collected. Your second piece of RNA treated tissue under this parent number would be K2300003_20); see example Worksheet at the beginning of this section.*

49 During clean up, place RNA tubes on a rocker within cold room (4 °C) and let rock Overnight

8h

50 **DAY 2**: the following day, again prep work area with RNase Zap.

51 Using a very small, tipped pipette, remove all RNAlater from tube.

Note

Tip: If necessary, RNAlater tissue can be stored at -20 °C for up to a week with RNAlater.

52 Move sample to -80 °C storage.

Preservation in 4% paraformaldehyde (PFA) (make fresh or use within 4 days, keep cold): Fixation

1d

53 Using regular forceps, gently place cut tissue selections in either a 2mL or 5mL tube with the fixative(depending on the room needed for the number of allocated pieces).
NOTE: As an alternative, neutral buffered formalin can also be used for fixation and in this case fixation is at room temperature,overnght

54 Place lid and temporarily store in Wet Ice container until all processing is complete.

55 Document sample types and number of pieces per tube (if using more than one tube, make sure to document tube number on both tube and worksheet).

56 During clean up, place 4% PFA tubes on a rocker within cold room (4 °C) and let incubate Overnight (12- 24:00:00).

1d

57 **DAY 2**: Replace 4% PFA with PBS.

Note

Caution: PFA is biohazardous, take care to discard 4% PFA in allocated biohazard container

57.1 Gently remove each piece of tissue using forceps and rinse thoroughly in a small dish of PBS.

Note

Alternative Rinsing Technique: Pour contents of 4% PFA tube into a small dish with a strainer
(possibly an empty FFPE cassette) and rinse with PBS using a pipette.

57.2 Gently place rinsed tissue back in tube prefilled with PBS.

Note

Tip: Same tube in which tissue was fixed can be re-used, however, make sure to indicate this
change of solution on the tube by crossing out “4% PFA” and writing “PBS.”

58 Document Fixation Stop Date/Time (amount of time specimen was in 4% PFA) on *Worksheet.*

59 Place tissue in PBS tubes on a rocker within cold room (4 °C) and let rock for at least 24 hours. Solution can be changed a few times to ensure residual PFA is washed out.

60 **DAY 3**: After specimens have rinsed in PBS they can be processed for either cryopreservation using sucrose to make a fixed frozen block (FFB) or prepared for paraffin embedding to make a paraffin block (PB).

Processing Fixed Frozen Blocks (FFB)

4d

61 Assign unique Specimen Label (*derivative of parent label*) for each FFB – *using our nomenclature, on the Worksheet, put a square around the next consecutive number after the O.C.T. blocks to indicate “Fixed Frozen Block” (i.e., K2300003_4FFB - This is your first piece of fixed frozen tissue. Your
second piece of fixed frozen tissue under this parent number would be K2300003_5FFB and so on); see example Worksheet at the beginning of this section.*

62 Label new 2mL tube with Specimen Label and “sucrose”.

63 Fill tubes with prepared sucrose (reference *Supply List – Section (D) Processing Liquids*).

64 Place assigned tissue in “sucrose” tube.

65 Document sample type on Worksheet.

66 Place new sucrose tubes on a rocker within cold room (4 °C) and let rock for around 1- 96:00:00 until specimen sinks to bottom of tube. 4d

67 Once specimen sinks, freeze in O.C.T. using instructions for processing (refer section "**Fresh Frozen Tissue (FB)**".

Processing Paraffin Blocks (PB) 8h

68 Assign unique Specimen Label (*derivative of parent label*) for each PB – *using our nomenclature, on the Worksheet, put a triangle around the next consecutive number after the FFB blocks to indicate “Paraffin Block” (i.e., K2300003_6PB - This is your first piece of fixed paraffin tissue. Your second piece of fixed paraffin tissue under this parent number would be K2300003_7PB and so on.); see example Worksheet at the beginning of this section (_7PB not shown).*

69 Label new 2mL tube with Specimen Label and “30% EtOH”.

70 Fill tubes with prepared 30% EtOH (reference Supply List –Section (D) Processing Liquids).

71 Place assigned tissue in “30% EtOH” tube.

72 Document sample type on Worksheet.

73 Place new 30% EtOH tubes on a rocker within cold room (4 °C) and let rock for several hours to Overnight . 8h

74 After several hours to Overnight remove 30% EtOH from tube using small pipette (leaving tissue in place).

75 Replace 30% EtOH with 70% EtOH (reference Supply List –Section (D) Processing Liquids for 70% EtOH recipe).

76 Cross out 30% EtOH and write 70% EtOH on tube.

77 Place new 70% EtOH tubes on a rocker within cold room (4 °C) until ready to make paraffin blocks.

78 Place tissue in labeled paraffin cassettes.

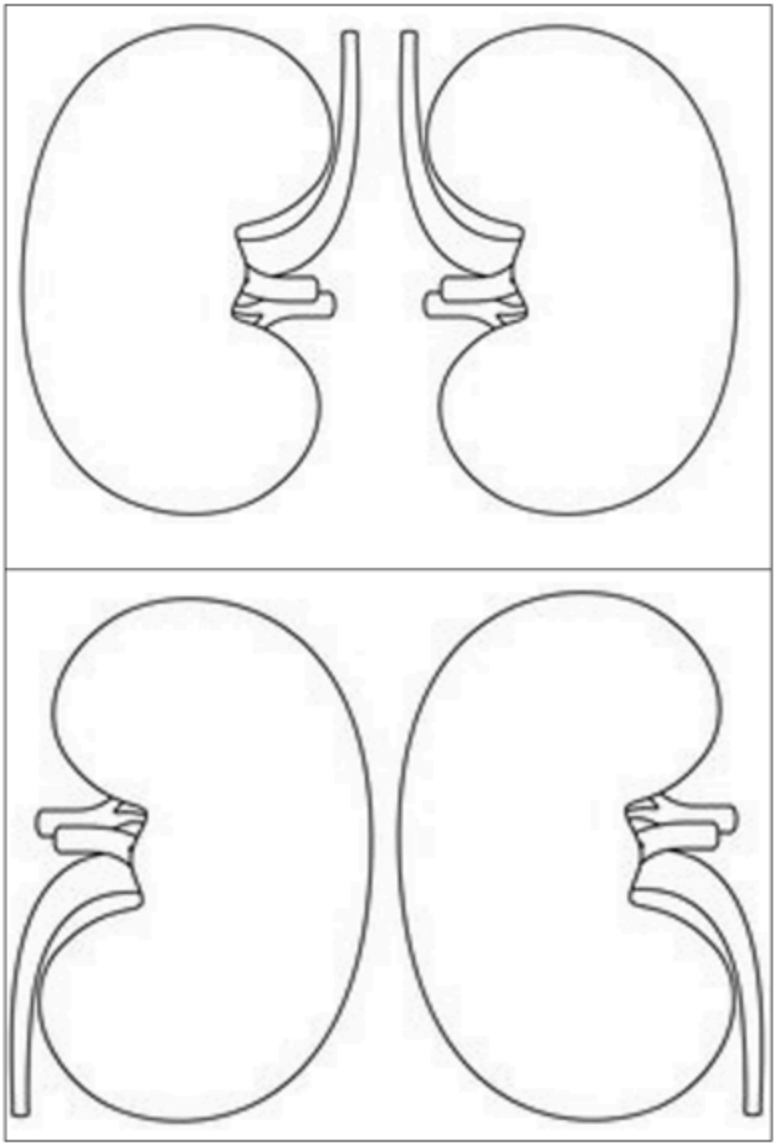
79 Store cassettes in beaker of 70% EtOH at 4 °C .

80 Submit labeled cassettes for paraffin embedding to the histology core facility.

PPID:	Date/Time of Extubation:	Left OPO: Arrived in Lab:	Kidney (cm):
	Date/Time of Profusion:	Total Transport Time (min):	Ureter (cm):
Date:	Warm Ischemic Time (min):	Processing Start Time:	Fixation Stop Date/Time:
Procedure: Deceased Donor Kidney	Cold Ischemic Time (min):	Processing Stop Time:	Total Fixation Time (min):
Laterality: ☐Right ☐Left	Total Ischemic Time (min):	Total Processing Time (min):	Processor's Initials:

Warm Ischemic Time = Elapsed time from Date/Time of Extubation to Date/Time of Perfusion
Cold Ischemic Time = Elapsed time from Date/Time of Perfusion to Processing Start Time
Total Ischemic Time = Warm Ischemic Time + Cold Ischemic Time
Total Fixation Time (FFB & PB only) = Elapsed Time from Processing Start Time to Fixation Stop Date/Time

Specimen Label	K23000	K23000	K23000		K23000		K23000		K23000		K23000	K23000
Specimen Location	Distal Ureter Start Time: Stop Time:	Proximal Ureter Start Time: Stop Time:	Region A ↑ ↓ mid ant post Start Time:	End Time:	Region B ↑ ↓ mid ant post Start Time:	End Time:	Region C ↑ ↓ mid ant post Start Time:	End Time:	Region D ↑ ↓ mid ant post Start Time:	End Time:	Pelvis Start Time: End Time:	Papilla Start Time: End Time:
O.C.T. (FB) ○	1	1	1	9	1	9	1	9	1	9	1	1
	2	2	2	10	2	10	2	10	2	10	2	2
Sucrose (FFB) □	3	3	3	11	3	11	3	11	3	11	3	3
	4	4	4	12	4	12	4	12	4	12	4	4
EtOH (PB) △	5	5	5	13	5	13	5	13	5	13	5	5
	6	6	6	14	6	14	6	14	6	14	6	6
Light Sheet (LS) ▭	7	7	7	15	7	15	7	15	7	15	7	7
	8	8	8	16	8	16	8	16	8	16	8	8
4% PFA (Specimen ID assigned later for 4% PFA samples)	>	>	Tube 1		Tube 1		Tube 1		Tube 1		>	>
	>	>	Tube 2		Tube 2		Tube 2		Tube 2		>	>
	>	>	Tube 3		Tube 3		Tube 3		Tube 3		>	>
LN2	17	17	17		17		17		17		17	17
	18	18	18		18		18		18		18	18
RNALater	19	19	19		19		19		19		19	19
	20	20	20		20		20		20		20	20
Specimen #1 (PBS)	21	21	21		21		21		21		21	21
Specimen #2 (PBS)	22	22	22		22		22		22		22	22



Protocol references

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