



Apr 05, 2024

Preparation, Processing and Preservation of Surgical Kidney Resections for Multiomic Studies

 [Nature Communications](#)

DOI

<https://dx.doi.org/10.17504/protocols.io.261ge56zdg47/v1>

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KPMP

Human BioMolecular Atl...

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DOI: <https://dx.doi.org/10.17504/protocols.io.261ge56zdg47/v1>

External link: <https://doi.org/10.1038/s41467-025-60435-8>



Protocol Citation: Sanjay Jain, Amanda Knoten, Kristy Conlon 2024. Preparation, Processing and Preservation of Surgical Kidney Resections for Multiomic Studies. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.261ge56zdg47/v1>

Manuscript citation:

McLaughlin, L., Zhang, B., Sharma, S. *et al.* Three dimensional multiscalar neurovascular nephron connectivity map of the human kidney across the lifespan. *Nat Commun* **16**, 5161 (2025). <https://doi.org/10.1038/s41467-025-60435-8>

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Protocol status: Working

We use this protocol and it's working

Created: February 21, 2024

Last Modified: April 05, 2024

Protocol Integer ID: 97854

Keywords: Kidney, Tissue preservation, Tissue processing, Ureter, Atlas, HuBMAP, KPMP, using kidney tissue procurement, kidney precision medicine project, human biomolecular atlas program, kidney tissue procurement as an example, preservation of surgical kidney resection, surgical kidney resection, multiomic studies multiomic technology, multiple tissue type, various atlasing project, detailed pipeline, end detailed pipeline, human sample, donor patient consent, donor, living donor patient consent, generating multidimensional data, tissue, high quality sample, multidimensional data

Funders Acknowledgements:

NIH

Grant ID: U54DK134301

NIH

Grant ID: U01DK114933

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 living donor patient consent to tissue procurement and processing that has been extensively tested on various atlasing 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.

Safety warnings

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

Before start

Please review the preparation materials in each section before starting.



Preparation: Obtaining Consent and Preparing to Collect the Kidney

- 1 Identify the potential nephrectomy case.
- 2 Request permission from the urologist to speak with the patient about possible consent.
- 3 Identify an opportunity to make initial contact with the patient, whether it be over the phone, at a preop appointment, or the day of the surgery.
- 4 Obtain consent from the patient.
- 5 Notify the urologist of the patient's consent so they will be aware that you will be retrieving and transporting the kidney to the gross room once it is removed.
- 6 Notify pathology/gross room of the scheduled date/time of the nephrectomy.
- 7 Determine what additional information or items the gross room will need to accompany the kidney, such as:
 1. Requisition for the pathology assessment
 2. Patient Labels
 3. Documentation confirming that research consent has been obtained
 4. Etc.

Note

Tip: Hospital issued scrubs will be needed to enter the operating room, so make arrangements to have those ahead of time.

Preparation: Day of the Nephrectomy

- 8 Prepare supplies (see next section Preparation: Before Kidney Arrival).



- 9 Once the surgery begins, make contact with the circulating nurse in the assigned operating room (via phone or in person):
 - 9.1 Let him/her know that you will be transporting the kidney to pathology/gross room immediately upon removal.
 - 9.2 Provide a list of the items you will need from the operating room to accompany the kidney to the gross room (i.e., requisition, patient labels, etc.).
 - 9.3 If possible, ask the nurse to document the *Clamp Time/Last Staple Time* (you will need this later to calculate the warm ischemic time).
 - 9.4 Make certain the nurse knows that you will be taking the kidney fresh and 🧊 On ice (No formalin).

Note

Tip: If possible, leave a cooler with ice in the operating room so the kidney can still immediately be placed 🧊 On ice in the event that you are unable to be there at the time of kidney removal. This will help ensure tissue preservation.

- 9.5 Make certain the nurse has a good contact phone number for you and request a phone call when the kidney is getting ready to be removed from the body.

Note

Tip: Have your name and contact number easily legible on the cooler for easy reference.

- 9.6 Before leaving the operating room, document the following on the Worksheet (find copy of Worksheet below):

i. *Clamp Time/Last Staple Time*

ii. *Time out of body*

iii. *Time on ice* 🧊 On ice

iv. *Left OR Time*



PPID:	Clamp Time/Last Staple Time:	Processing Start Time:	Kidney (cm):
Date:	Time out of body: Time on ice:	Processing Stop Time:	Ureter (cm):
Procedure: Nephrectomy	Left OR Time:	Warm Ischemic Time (min):	Fixation Stop Date/Time:
Laterality: ☐ Right ☐ Left	Arrived in Gross Room Time:	Cold Ischemic Time (min):	Total Fixation Time (min):
	Total Transport Time:	Total Ischemic Time (min):	Processor's Initials:

- 9.7 Immediately bring the fresh kidney  On ice to the gross room to begin processing.

Note

- *Tip: If possible, have everything set up in the gross room ahead of time to cut down on processing time and thus tissue preservation*
- *Tip: If possible, coordinate with a partner to fill the **LN2 Transport Container** and bring the **LN2 Transport Container** and all other **Supplies** (if not already set up) to the gross room at the same time the kidney is leaving the operating room. This can be coordinated via phone or text and ensures that there is plenty of LN2 to last throughout the processing.*

Preparation: Before Kidney Arrival

- 10 Prepare *Worksheet* with initial information (find copy of Worksheet below):

- PPID
- Date (of collection)
- Laterality

PPID:	Clamp Time/Last Staple Time:	Processing Start Time:	Kidney (cm):
Date:	Time out of body: Time on ice:	Processing Stop Time:	Ureter (cm):
Procedure: Nephrectomy	Left OR Time:	Warm Ischemic Time (min):	Fixation Stop Date/Time:
Laterality: ☐ Right ☐ Left	Arrived in Gross Room Time:	Cold Ischemic Time (min):	Total Fixation Time (min):
	Total Transport Time:	Total Ischemic 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	Proximal Ureter	Region A	Region B	Region C	Tumor	Pelvis	Papilla/Calyx
	Start Time: Stop Time:	Start Time: Stop Time:	Start Time: End Time: ant post	Start Time: End Time: ant post	Start Time: End Time: ant post	Start Time: End Time:	Start Time: End Time:	Start Time: End Time:

- 11 Label preliminary collection cassettes and tubes using the unique Specimen Labels prepared on the *Worksheet*. The table below indicates a good 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	I
	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	Tumor	Pelvis	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	4	1	1
	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 tubes (for 4% PFA samples, pre-fill $\frac{3}{4}$ 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	1	1
	**5mL tubes (for 4% PFA			1	1	1			



	A	B	C	D	E	F	G	H	I
	samples, pre-fill $\frac{3}{4}$ 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))								
	2mL tubes (for Fresh Frozen LN2 – no fluid in tubes)	1	1	1	1	1	1	1	1
	1.5mL tubes (for RNA samples, pre-fill $\frac{3}{4}$ of the way with RNALater)	1	1	1	1	1	1	1	1
	Optional 5mL tubes (for additional fresh samples, fill tube $\frac{3}{4}$ 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)

- 12 Prefill the pre-labeled tubes (3/4 full) with 4%PFA, PBS or RNA for faster processing once kidney arrives.
 - 12.1 Reference *Supply List_Processing Kidney Tissue Section (D) Processing Liquids* for information on liquids needed and how to prepare solutions.
 - 12.2 Immediately place prelabeled 4% PFA and PBS containers in wet ice.
- 13 Prepare processing area
 - 13.1 Clean work area, prepare as if working in sterile conditions.

13.2 Treat any area where RNA samples will be processed with RNase Zap to prevent RNA degradation.

14 Gather Supplies:

a) **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)

b) **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.

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

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

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

f) Additional **RNA & RNase Zap** for additional RNA collections

g) **O.C.T**

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

i) Processing Tools – **clipboard with Worksheet_Live 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

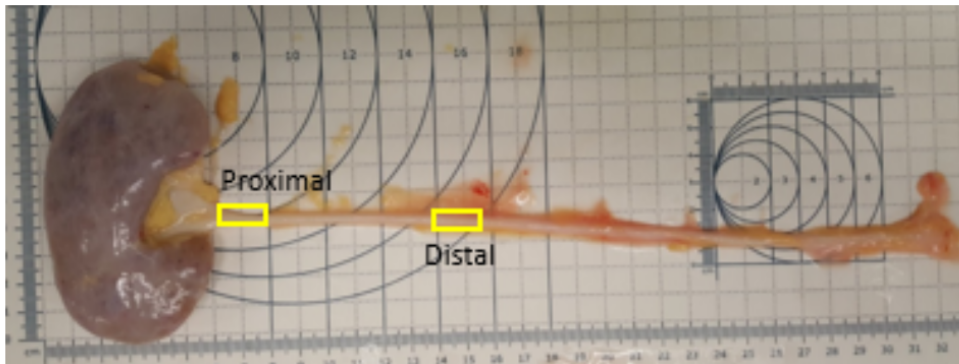
15 Document Arrived in Gross Room Time on Worksheet.

16 Work with the PA in the gross room to prep kidney for dissection:

- 16.1 Prepare cutting board to receive kidney.
- 16.2 Trim surrounding fat and large vessels to expose kidney and ureter.
- 16.3 Orient the kidney and attached ureter on cutting board.
- 16.4 Identify posterior and anterior aspects.
- 16.5 Identify upper, mid, and lower poles.
- 16.6 Identify pelvis.
- 16.7 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
- 16.8 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.

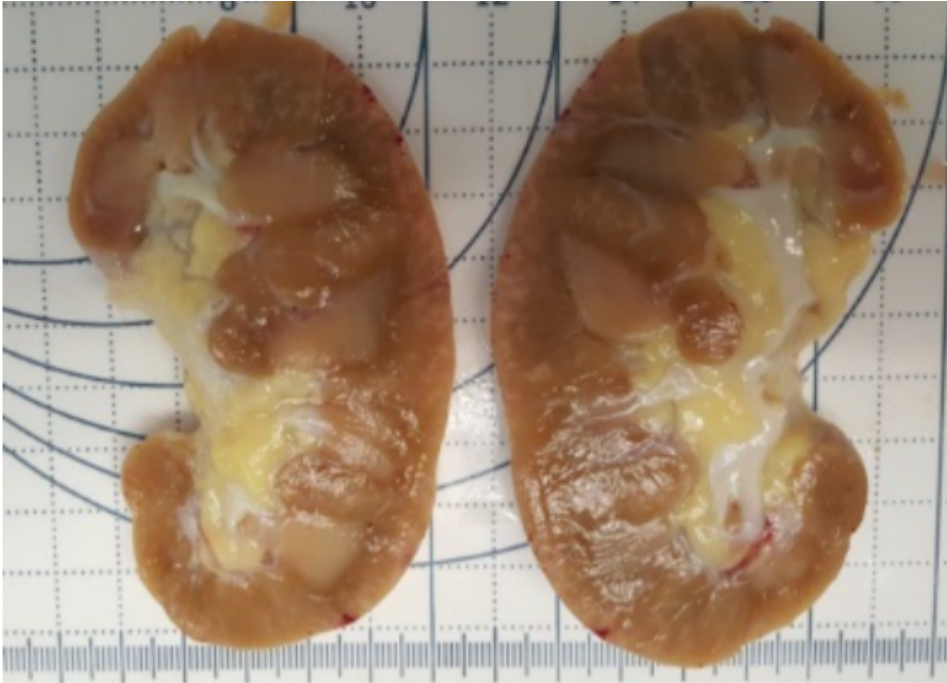


- 16.9 Pour LN2 in cooler up to the top of metal embedding well (or block). Do not cover top surface of block. LN2 may need to be replenished several times while processing.

Processing: Kidney Dissection

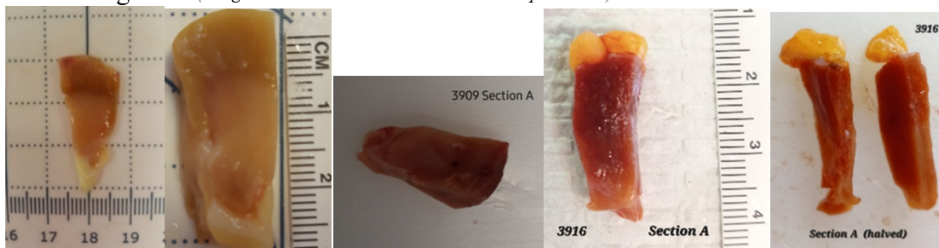
- 17 Distal Ureter:
- 17.1 Document *Start Time* for Distal Ureter and overall *Processing Start Time* on *Worksheet*.
- 17.2 Obtain approximately 1-3cm of distal ureter.
- 17.3 Cut into roughly 2mm cross sections and allocate for desired processing.
- 17.4 Process Distal Ureter segments following **Processing Instructions for different types of preservation options** found below.
- 17.5 Document *Stop Time* and sample types collected for distal ureter.
- 18 Proximal Ureter:
- 18.1 Document *Start Time*.

- 18.2 Obtain approximately 1-3cm of proximal ureter (*proximal ureter boundary is at UPJ*).
- 18.3 Cut into roughly 2mm cross sections and allocate for desired processing.
- 18.4 Process Proximal Ureter segments following **Processing Instructions for different types of preservation options** found below.
- 18.5 Document Stop Time and sample types collected for proximal ureter.
- 19 Bisect kidney on the longest axis.



- 19.1 Take picture of open kidney (*include ruler if not on cutting board*).
- 19.2 Examine kidney for any gross abnormalities and document.

- 19.3 Select healthy appearing lobe(s) for dissection (the lobe typically includes an outer cortex and underlying medullary pyramid).
- 19.4 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*).

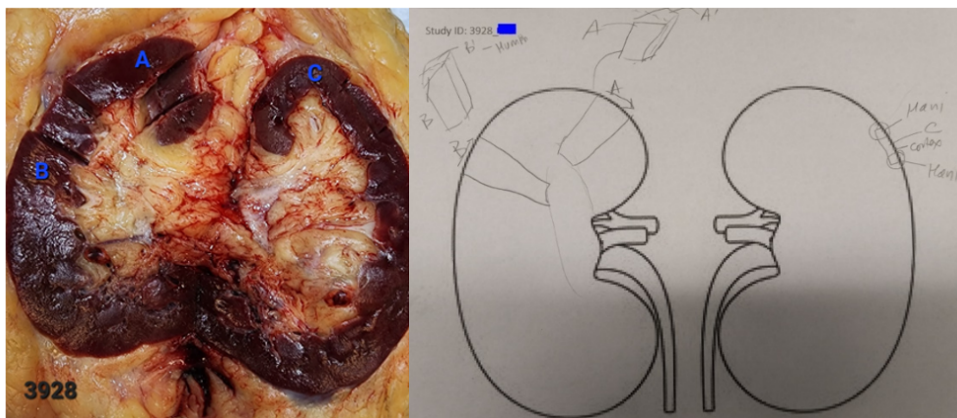


- 20 Region A:
- 20.1 Document *Start Time*, pole location, and anterior or posterior.
- 20.2 Take a picture of Region A (*include ruler*).
- 20.3 Cut desired sections from lobe and designate processing method for each piece.



- 20.4 Process Region A pieces following **Processing Instructions for different types of preservation options** found below.

- 20.5 Document Stop Time and sample types collected for Region A.
- 21 Repeat process for Regions B, C ...
- 22 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*).
- 23 On kidney drawing, found on second page of *Worksheet*, document location of collected lobes as a reference for cut regions shown in picture.



Processing: Final Documentation

- 24 Document *Processing Stop Time*.
- 25 Confirm that each sample is accounted for and correctly marked on *Worksheet*.
- 26 Calculate & document *Total Transport Time* in minutes (Elapsed time between *Left OR Time* to *Arrived in Gross Room Time*).
- 27 Calculate & document *Warm Ischemic Time* in minutes (Elapsed time between *Clamp Time/Last Staple Time* to Time  On ice).

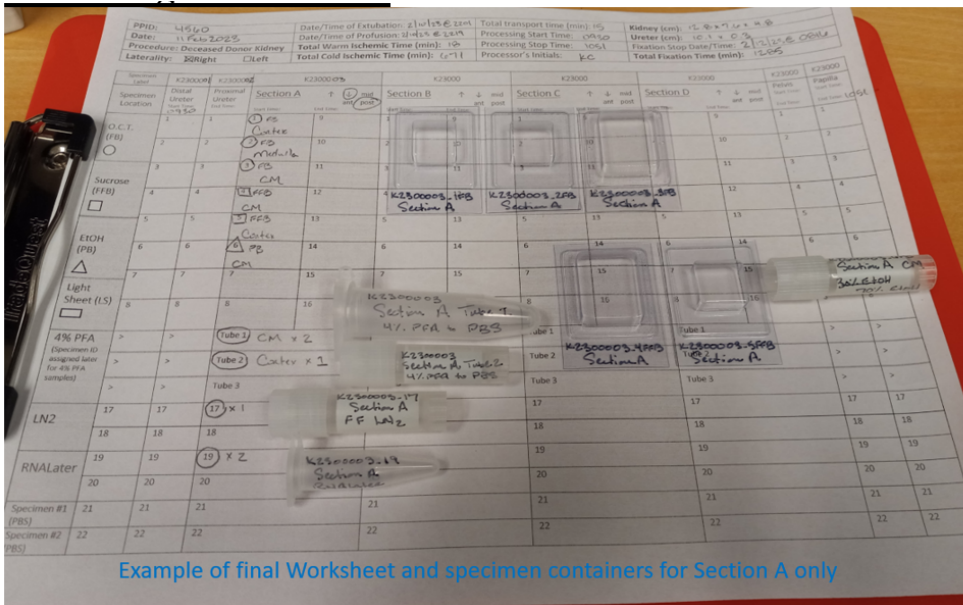
**Note**

Tip: If a Clamp Time/Last Staple Time is not available, count back 30 minutes from the Time out of Body (or kidney removal)

- 28 Calculate & document *Cold Ischemic Time* in minutes (Elapsed time from *Time*  On ice to *Processing Start Time*).
- 29 Calculate & document *Total Ischemic Time* in minutes (*Total of Warm Ischemic Time* and *Cold Ischemic Time*).
- 30 Calculate and document *Total Processing Time* in minutes (Elapsed time from *Processing Start Time* to *Processing Stop Time*).
- 31 Document *Processor's Initials*.
- 32 The following day, when the 4% PFA is changed to PBS, document the *Fixation Stop Date/Time* on *Worksheet*.
- 33 Calculate and document *Total Fixation Time* in minutes (Elapsed Time from *Processing Start Time* to *Fixation Stop Date/Time*).

Processing Instructions:

34



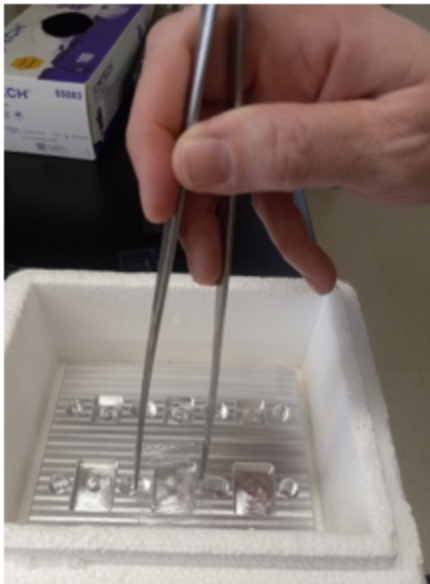
Fresh Frozen Tissue (FB):

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



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

- 37 Place O.C.T. covered tissue strategically in labeled cassette.
- 38 Squirt additional O.C.T. into cassette well until tissue is completely covered.
- 39 With large forceps, carefully place filled cassette onto embedding well or metal block within the LN2 cooler.
- 40 Cover cooler and monitor carefully.
- 41 Once block is completely frozen, use large forceps to remove frozen cassette from cooler and place on bed of powdered dry ice.



- 42 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.*
- 43 When finished processing place in labeled resealable bag for protection.

- 44 Sample is ready for  -80 °C storage.



Flash Fresh Frozen LN2 Samples:

- 45 Using regular forceps, gently place cut tissue selection in empty labeled tube and place lid.
- 46 With large forceps place tube directly in LN2 within the cooler.
- 47 Cover cooler and monitor carefully.
- 48 Once completely frozen, use large forceps to remove tube from cooler and place on bed of powdered dry ice.



49 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.*

50 Sample is ready for  -80 °C storage.

RNA Samples:

8h

51 Using regular forceps, gently place cut tissue selection in labeled tube (filled $\frac{3}{4}$ of the way with RNAlater).

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

53 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.*

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

8h



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

56 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.

57 Move sample to  -80 °C storage.



4% PFA Samples (make fresh or use within 4 days, keep cold):

2d

- 58 Using regular forceps, gently place cut tissue selections in either a 2mL or 5mL tube (depending on the room needed for the number of allocated pieces).
- 59 Place lid and temporarily store in Wet Ice container until all processing is complete.
- 60 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*).
- 61 During clean up, place 4% PFA tubes on a rocker within cold room ( 4 °C) and let incubate overnight (12-  24:00:00).
- 62 **DAY 2:** Replace 4% PFA with PBS.

1d

Note

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

- 62.1 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.

- 62.2 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."



- 63 Document Fixation Stop Date/Time (amount of time specimen was in 4% PFA) on *Worksheet*.
- 64 Place tissue in PBS tubes on a rocker within cold room ( 4 °C) and let rock for at least  24:00:00 . Solution can be changed a few times to ensure residual PFA is washed out. 1d
- 65 **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

- 66 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.*
- 67 Label new 2mL tube with Specimen Label and "sucrose".
- 68 Fill tubes with prepared sucrose (reference *Supply List – Section (D) Processing Liquids*).
- 69 Place assigned tissue in "sucrose" tube.
- 70 Document sample type on *Worksheet*.
- 71 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
- 72 Once specimen sinks, freeze in O.C.T. using instructions for processing (refer section "**Fresh Frozen Tissue (FB)**" above).



Processing Paraffin Blocks (PB)

8h

- 73 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).*
- 74 Label new 2mL tube with Specimen Label and “30% EtOH”.
- 75 Fill tubes with prepared 30% EtOH (reference *Supply List – Section (D) Processing Liquids*).
- 76 Place assigned tissue in “30% EtOH” tube.
- 77 Document sample type on *Worksheet*.
- 78 Place new 30% EtOH tubes on a rocker within cold room ( 4 °C) and let rock for several hours to  Overnight .
- 79 After several hours to overnight remove 30% EtOH from tube using small pipette (leaving tissue in place).
- 80 Replace 30% EtOH with 70% EtOH (reference *Supply List – Section (D) Processing Liquids* for 70% EtOH recipe).
- 81 Cross out 30% EtOH and write 70% EtOH on tube.
- 82 Place new 70% EtOH tubes on a rocker within cold room ( 4 °C) until ready to make paraffin blocks.
- 83 Place tissue in labeled paraffin cassettes.

8h

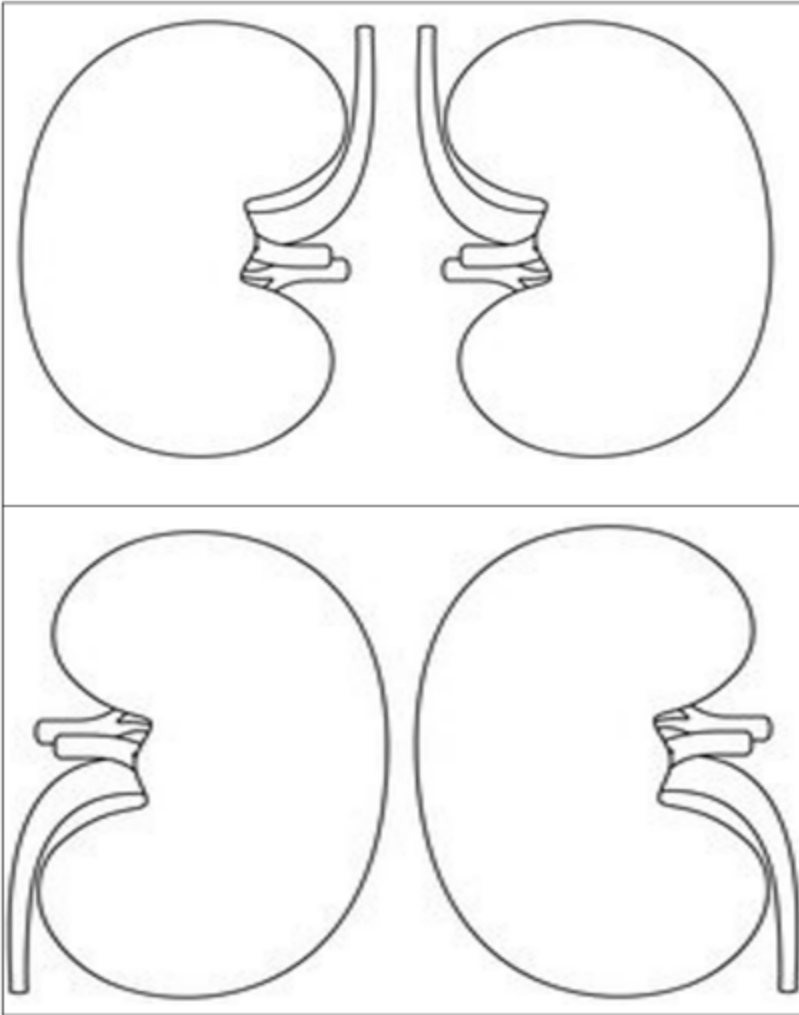
84 Store cassettes in beaker of 70% EtOH at 4 °C .

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

PPID:		Clamp Time/Last Staple Time:		Processing Start Time:		Kidney (cm):	
Date:		Time out of body:		Processing Stop Time:		Ureter (cm):	
Procedure: Nephrectomy		Left OR Time:		Warm Ischemic Time (min):		Fixation Stop Date/Time:	
Laterality: C/Right C/Left		Arrived in Gross Room Time:		Cold Ischemic Time (min):		Total Fixation Time (min):	
		Total Transport Time:		Total Ischemic Time (min):		Processor's Initials:	

Warm Ischemic Time = Elapsed time between Clamp Time/Last Staple Time to Time on ice
Cold Ischemic Time = Elapsed time from Time on ice 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	
	Distal Ureter	Proximal Ureter	Region A		Region B		Region C		Tumor		Pelvis		Papilla/Calyx			
Specimen Location	Start Time	Stop Time	Start Time	End Time	Start Time	End Time	Start Time	End Time	Start Time	End Time	Start Time	End Time	Start Time	End Time	Start Time	End Time
O.C.T. (FB) ○	1	1	1	9	1	9	1	9	1	1	1	1	1	1	1	1
	2	2	2	10	2	10	2	10	2	2	2	2	2	2	2	2
Sucrose (FFB) □	3	3	3	11	3	11	3	11	3	3	3	3	3	3	3	3
	4	4	4	12	4	12	4	12	4	4	4	4	4	4	4	4
EtOH (PB) △	5	5	5	13	5	13	5	13	5	5	5	5	5	5	5	5
	6	6	6	14	6	14	6	14	6	6	6	6	6	6	6	6
Light Sheet (LS) □	7	7	7	15	7	15	7	15	7	7	7	7	7	7	7	7
	8	8	8	16	8	16	8	16	8	8	8	8	8	8	8	8
4% PFA (Specimen ID assigned later for 4% PFA samples)	>	>	Tube 1		Tube 1		Tube 1		>	>	>		>	>	>	>
	>	>	Tube 2		Tube 2		Tube 2		>	>	>		>	>	>	>
	>	>	Tube 3		Tube 3		Tube 3		>	>	>		>	>	>	>
LN2	17	17	17		17		17		17		17		17		17	
	18	18	18		18		18		18		18		18		18	
RNALater	19	19	19		19		19		19		19		19		19	
	20	20	20		20		20		20		20		20		20	
Specimen #1 (PBS)	21	21	21		21		21		21		21		21		21	
Specimen #2 (PBS)	22	22	22		22		22		22		22		22		22	



PPID:



Protocol references

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