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Protocol status: Working
We use this protocol and it's working

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

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KPMP

Human BioMolecular Atlas Program (HuBMAP) Method Development Community

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ABSTRACT

Multomic 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).

PROTOCOL REFERENCES

Blue B. Lake, Rajasree Menon, Seth Winfree, Qiwen Hu, Ricardo Melo Ferreira, ... Michael T. Eadon, Pierre C. Dagher, Tarek M. El-Achkar, Kun Zhang, Matthias Kretzler, Sanjay Jain. An atlas of healthy and injured cell states and niches in the human kidney. *Nature* 619, 585–594 (2023). <https://doi.org/10.1038/s41586-023-05769-3>.

A multimodal and integrated approach to interrogate human kidney biopsies with rigor and reproducibility: guidelines from the Kidney Precision Medicine Project

Tarek M. El-Achkar, Michael T. Eadon, Rajasree Menon, Blue B. Lake, Tara K. Sigdel, Theodore Alexandrov, Samir Parikh, Guanshi Zhang, Dejan Dobi, Kenneth W. Dunn, Edgar A. Otto, Christopher R. Anderton, Jonas M. Carson, Jinghui Luo, Chris Park, Habib Hamidi, Jian Zhou, Paul Hoover, Andrew Schroeder, Marianinha Joanes, Evren U. Azeloglu, Rachel Sealfon, Seth Winfree, Becky Steck, Yongqun He, Vivette D'Agati, Ravi Iyengar, Olga G. Troyanskaya, Laura Barisoni, Joseph Gaut, Kun Zhang, Zoltan Laszik, Brad H. Rovin, Pierre C. Dagher, Kumar Sharma, Minnie M. Sarwal, Jeffrey B. Hodgin, Charles E. Alpers, Matthias Kretzler, and Sanjay Jain

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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 INSTRUCTIONS

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: [REDACTED]	Date/Time of Extubation: [REDACTED]	Left OPO: [REDACTED]	Kidney (cm): [REDACTED]
Date: [REDACTED]	Date/Time of Profusion: [REDACTED]	Total Transport Time (min): [REDACTED]	Ureter (cm): [REDACTED]
Procedure: Deceased Donor Kidney	Warm Ischemic Time (min): [REDACTED]	Processing Start Time: [REDACTED]	Fixation Stop Date/Time: [REDACTED]
	Cold Ischemic Time (min): [REDACTED]	Processing Stop Time: [REDACTED]	Total Fixation Time (min): [REDACTED]
Laterality: <input checked="" type="checkbox"/> Right <input type="checkbox"/> Left	Total Ischemic Time (min): [REDACTED]	Total Processing Time (min): [REDACTED]	Processor's Initials: [REDACTED]

- 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	↑ ↓ mid ant post	Region B	↑ ↓ mid ant post	Region C	↑ ↓ mid ant post
Start Time:	Start Time:	Start Time:	Start Time:	End Time:	Start Time:	End Time:	Start Time:	End Time:
Stop Time:	Stop Time:	Stop Time:	Stop Time:	Stop Time:	Stop Time:	Stop Time:	Stop Time:	Stop 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

A	B	C	D	E	F	G	H
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
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.

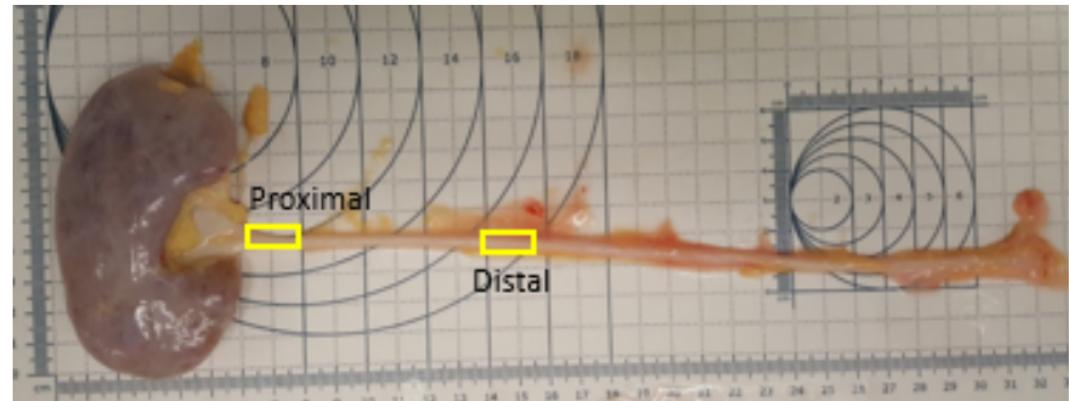
11.5 Identify upper, mid, and lower poles.

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.

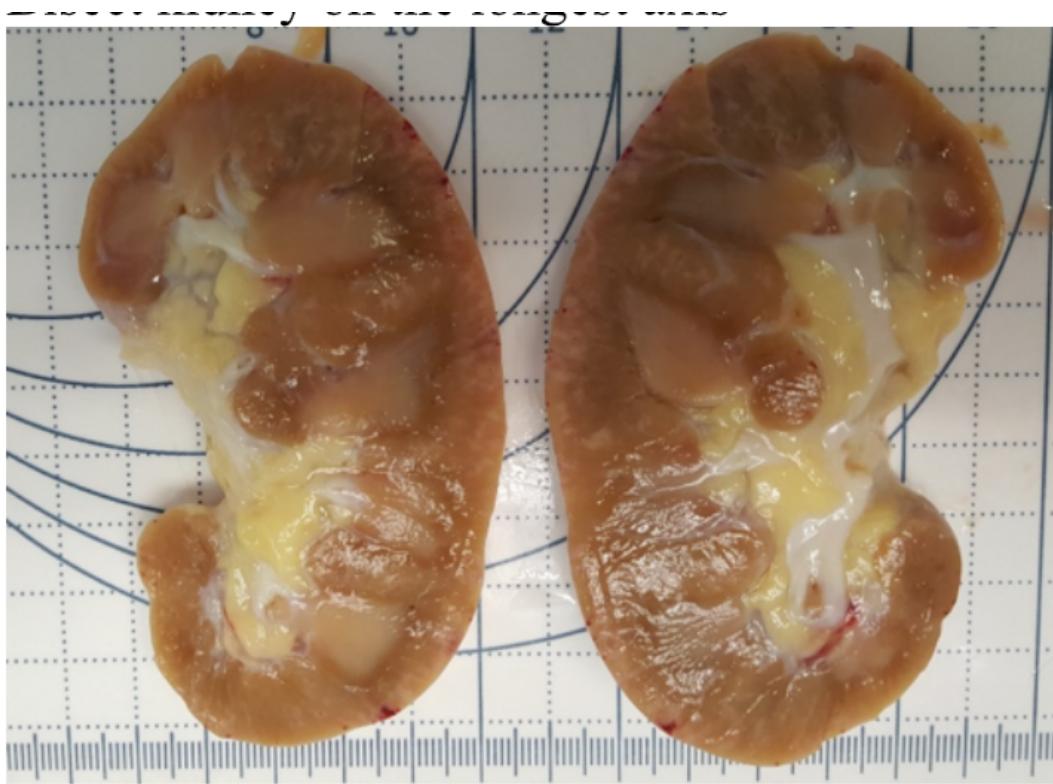
12.5 Document *Stop Time* and sample types collected for distal ureter.

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.

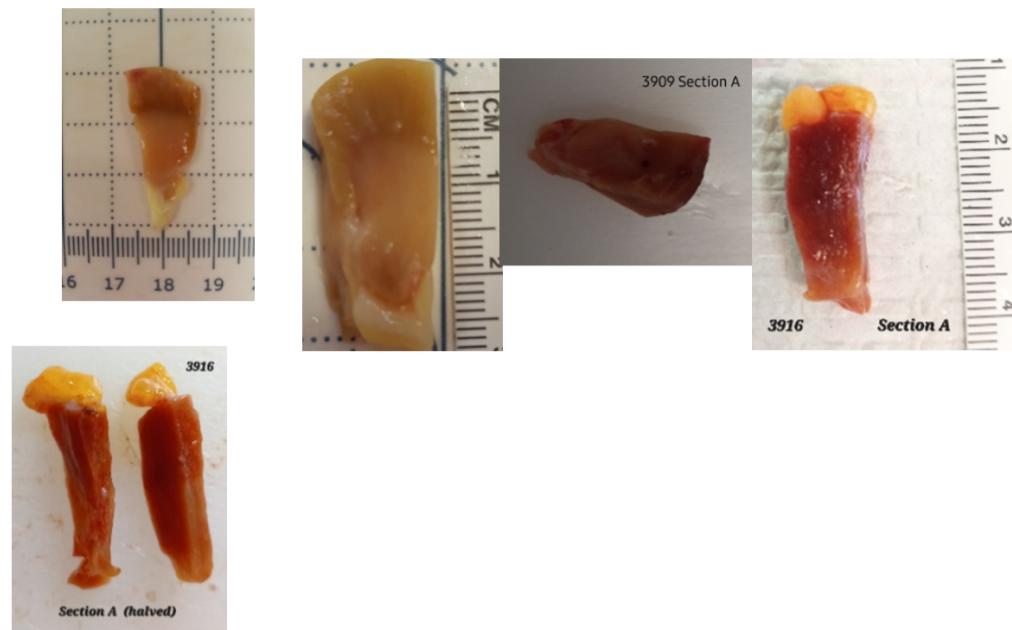


14.1 Take picture of open kidney (*include ruler if not on cutting board*).

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

PPID:		445-0	Date/Time of Extraction:		11-15-2023, 10:00 AM	Total Transport Time (using 165)		165
Date:		11-15-2023	Date/Time of Preparation:		11-15-2023, 10:00 AM	Processing Start Time:		10:00 AM
Processor:		Decreasen Donor Radiney	Total Mean Ischemic Time (min):		10	Processing Stop Time:		10:00 AM
Laterality:		Right	Specimen ID:		K2300003	Processor's Initials:		YC
Specimen Location	Specimen ID	Date/Time of Extraction	Processor	Date/Time of Preparation	Total Mean Ischemic Time (min)	Section A	Section B	Section C
U.C.T. (FB)	1	11-15-2023, 10:00 AM	Decreasen	11-15-2023, 10:00 AM	10	1	2	3
Surround (FB)	2	11-15-2023, 10:00 AM	Carter	11-15-2023, 10:00 AM	10	4	5	6
	3	11-15-2023, 10:00 AM	Decreasen	11-15-2023, 10:00 AM	10	7	8	9
	4	11-15-2023, 10:00 AM	CM	11-15-2023, 10:00 AM	10	10	11	12
EtOH (FB)	5	11-15-2023, 10:00 AM	CM	11-15-2023, 10:00 AM	10	13	14	15
Light Sheet (HS)	6	11-15-2023, 10:00 AM	Carter	11-15-2023, 10:00 AM	10	16	17	18
	7	11-15-2023, 10:00 AM	CM	11-15-2023, 10:00 AM	10	19	20	21
4% PFA (Specimen ID assigned later for all PFA samples)	8	11-15-2023, 10:00 AM	Tube 1 CM x 2	11-15-2023, 10:00 AM	10	22	23	24
	9	11-15-2023, 10:00 AM	Tube 2 Carter x 2	11-15-2023, 10:00 AM	10	25	26	27
	10	11-15-2023, 10:00 AM	Tube 3	11-15-2023, 10:00 AM	10	28	29	30
EN2	11	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	31	32	33
	12	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	34	35	36
RNALater	13	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	37	38	39
	14	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	40	41	42
Specimen #1 (PBS)	15	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	43	44	45
Specimen #2 (PBS)	16	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	46	47	48
	17	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	49	50	51
	18	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	52	53	54
	19	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	55	56	57
	20	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	58	59	60
	21	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	61	62	63
	22	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	64	65	66
	23	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	67	68	69
	24	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	70	71	72
	25	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	73	74	75
	26	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	76	77	78
	27	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	79	80	81
	28	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	82	83	84
	29	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	85	86	87
	30	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	88	89	90
	31	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	91	92	93
	32	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	94	95	96
	33	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	97	98	99
	34	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	100	101	102
	35	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	103	104	105
	36	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	106	107	108
	37	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	109	110	111
	38	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	112	113	114
	39	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	115	116	117
	40	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	118	119	120
	41	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	121	122	123
	42	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	124	125	126
	43	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	127	128	129
	44	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	130	131	132
	45	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	133	134	135
	46	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	136	137	138
	47	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	139	140	141
	48	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	142	143	144
	49	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	145	146	147
	50	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	148	149	150
	51	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	151	152	153
	52	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	154	155	156
	53	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	157	158	159
	54	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	160	161	162
	55	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	163	164	165
	56	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	166	167	168
	57	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	169	170	171
	58	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	172	173	174
	59	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	175	176	177
	60	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	178	179	180
	61	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	181	182	183
	62	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	184	185	186
	63	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	187	188	189
	64	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	190	191	192
	65	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	193	194	195
	66	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	196	197	198
	67	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	199	200	201
	68	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	202	203	204
	69	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	205	206	207
	70	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	208	209	210
	71	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	211	212	213
	72	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	214	215	216
	73	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	217	218	219
	74	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	220	221	222
	75	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	223	224	225
	76	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	226	227	228
	77	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	229	230	231
	78	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	232	233	234
	79	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	235	236	237
	80	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	238	239	240
	81	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	241	242	243
	82	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	244	245	246
	83	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	247	248	249
	84	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	250	251	252
	85	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	253	254	255
	86	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	256	257	258
	87	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	259	260	261
	88	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	262	263	264
	89	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	265	266	267
	90	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	268	269	270
	91	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	271	272	273
	92	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	274	275	276
	93	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	277	278	279
	94	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	280	281	282
	95	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	283	284	285
	96	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	286	287	288
	97	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	289	290	291
	98	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	292	293	294
	99	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	295	296	297
	100	11-15-2023, 10:00 AM	Section A FF LNZ	11-15-2023, 10:00 AM	10	298	299	300

Fresh Frozen Tissue (FB): OCT embedding

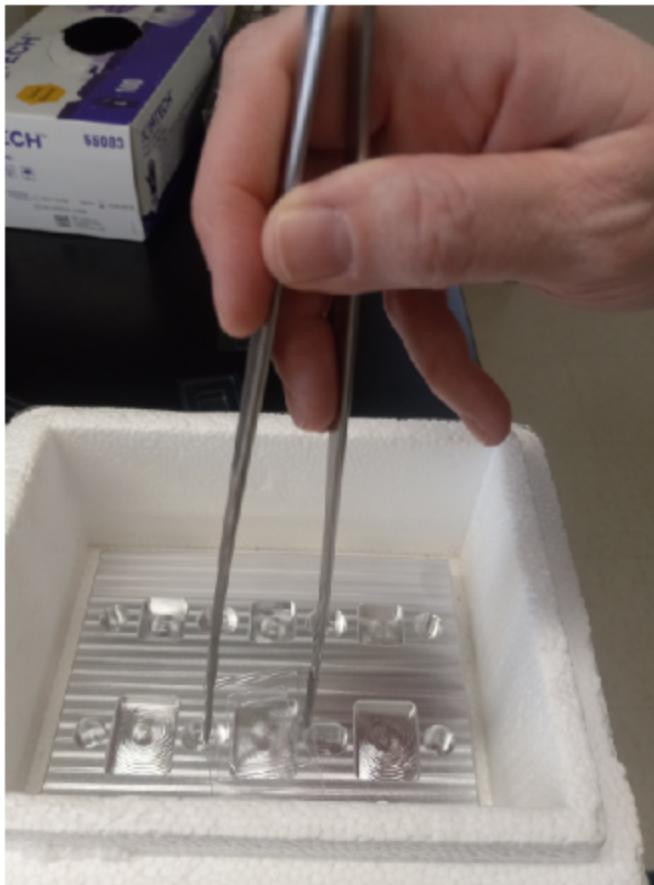
30 Squirt a quarter sized amount of O.C.T on a 4" x 4" square of paraffin film.



- 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 placing the metal block in the cooler or styrofoam container, fill the container with LN2 to up to 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 da... 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, overnight

- 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  1d  Overnight (12-  24:00:00).
- 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  4d until specimen sinks to bottom of tube.
- 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.

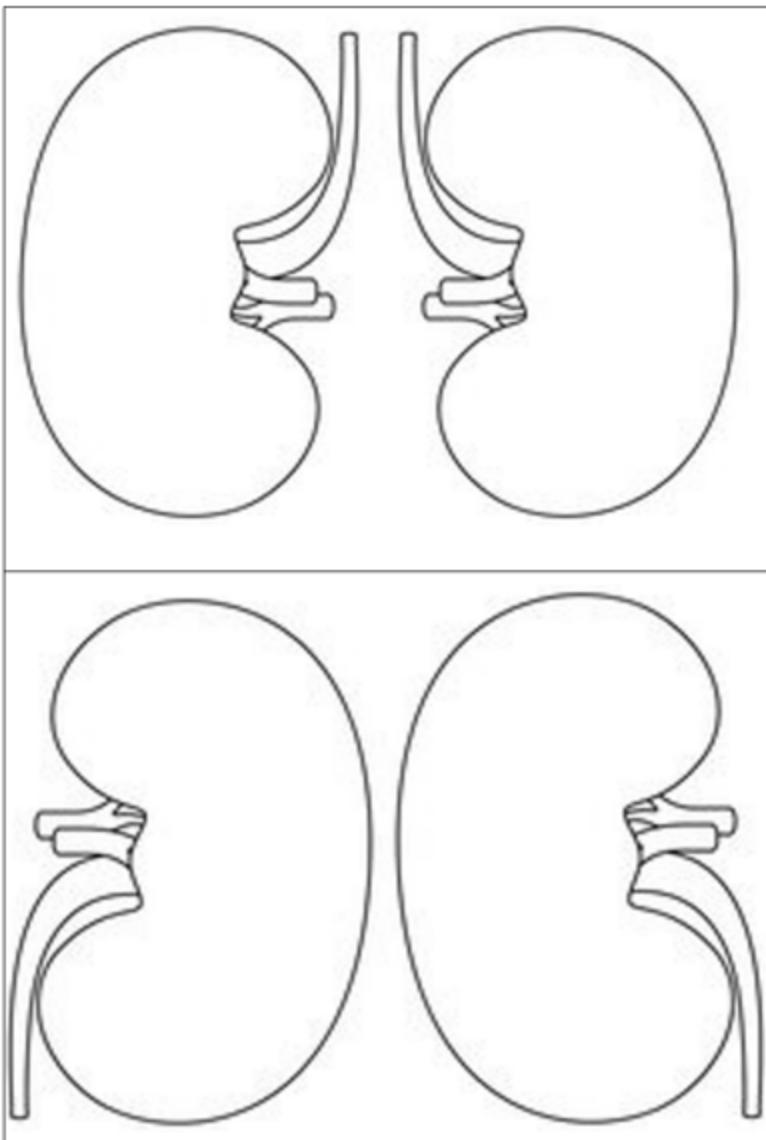
77 Place new 70% EtOH tubes on a rocker within cold room ( 4 °C) until ready to make paraffin blocks.

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:						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 Profusion 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 (min) = Total Ischemic Time + Elapsed Time from Processing Start Time to Fixation Stop Date/Time										
Specimen Label	K23000	K23000	K23000	K23000	K23000	K23000	K23000	K23000	K23000	K23000
Specimen Location	Distal Ureter	Proximal Ureter	Region A ↑ ↓ mid ant post	Region B ↑ ↓ mid ant post	Region C ↑ ↓ mid ant post	Region D ↑ ↓ mid ant post	Pelvis	Papilla		
O.C.T. (FB) ○	1	1	1 9	1 9	1 9	1 9	1	1	1	1
Sucrose (HRB) □	2	2	2 10	2 10	2 10	2 10	2	2	2	2
EtOH (PB) △	3	3	3 11	3 11	3 11	3 11	3	3	3	3
Light Sheet (LS) □	4	4	4 12	4 12	4 12	4 12	4	4	4	4
4% PFA (Specimen ID assigned later for 4% PFA samples)	5	5	5 13	5 13	5 13	5 13	5	5	5	5
LN2	6	6	6 14	6 14	6 14	6 14	6	6	6	6
7	7	7	7 15	7 15	7 15	7 15	7	7	7	7
8	8	8	8 16	8 16	8 16	8 16	8	8	8	8
17	17	17	17	17	17	17	17	17	17	17
18	18	18	18	18	18	18	18	18	18	18
19	19	19	19	19	19	19	19	19	19	19
20	20	20	20	20	20	20	20	20	20	20
Specimen #1 (PBS)	21	21	21	21	21	21	21	21	21	21
Specimen #2 (PBS)	22	22	22	22	22	22	22	22	22	22



PPD: