



**Sample Preparation and Tissue  
Mounting Instructions:  
Fresh frozen tissue sections**

### Summary of requirements

- *OCT-embedded tissue (Cryostat)*
- *Tissue Slides coated for tissue adhesion (Suggestions below):*
  - Superfrost Plus slides (Thermo Fisher Scientific)
  - Poly-L-Lysine (PLL) coated slides (Thermo Fisher Scientific)
- *Sections must be cut to 7-10 micron thickness max.*
- *Sections must be placed in the regions specified*
- *Shipping Manifest should be completed to identify specimens by Slide ID*

## 1. Tissue Freezing and Embedding

### Sample preparation

Immediately following harvesting of tissue, remove and absorb excess blood or solution with a rolled-up laboratory wipe. Immediately move to freezing and embedding step.

### Freezing and embedding protocol

Two freezing and embedding protocols are widely used for spatial omics studies with fresh frozen tissue. Both are acceptable for the AtlasXomics platform. If fresh tissue is available, simultaneous freezing and embedding may be preferred.

#### Option 1: Freezing followed by OCT embedding

- Prepare a metal beaker with isopentane and place it in a liquid nitrogen bath dewar for 5-10 mins
- Immerse the tissue into chilled isopentane for ~1 min or until completely frozen
- Place the tissue into a cryomold on dry ice, prefilled with prechilled OCT (4°C)
- Add prechilled OCT (4°C) to the cryomold completely submerging the tissue. Wait until the OCT completely solidifies and turns white. Make sure to avoid bubbles.

#### Option 2: Simultaneous freezing and OCT embedding

- Prepare a metal beaker with isopentane and place it in a liquid nitrogen bath dewar for 5-10 mins
- Place the tissue into the cryomold on dry ice, prefilled with prechilled OCT (4°C)
- Pour prechilled OCT(4°C) into a tissue embedding plastic mold until the OCT completely covers the tissue.
- Using forceps, transfer the plastic mold into the isopentane and liquid nitrogen bath without submerging. Wait until the OCT completely solidified and turns white.
- Note: A smaller tissue block is preferred as it can be frozen faster than a larger one (target < 1.0 cm<sup>3</sup>). Minimize freeze-thaw cycles as much as possible.

Store the OCT embedded tissue block in a sealed container at -80°C for long-term storage or immediately proceed to cryosectioning.

## 2. Cryosectioning

- Place the OCT compound-embedded tissue block into the cryotome and allow it to sit at  $-20^{\circ}\text{C}$  for at least 30 min prior to sectioning.
- Note: Recommended sectioning temperature is  $-20^{\circ}\text{C}$  for cryostat blade and  $-10^{\circ}\text{C}$  for the specimen head. Follow manufacturer's manual for detailed operations. Note: *adjusting temperature may improve sectioning performance for specific tissues.*
- Trim the OCT-embedded tissue block until the desired tissue region is exposed.
- Cut a  $10\ \mu\text{m}$  max section from the OCT block
  - Note: *AtlasXomics recommends collecting an additional 5-10 tissue sections (from either side of the section of interest), per tissue type, for optimization, but we are able to proceed with as few as 2-3 sections for valuable samples if a similar sample is available for optimization.*
- Avoid tissue cracking, curling and crumpling as this will diminish the performance of the platform. Optimize sectioning parameters on a practice block prior to cutting the sample of interest.

## 3. Tissue placement for standard 1x3" pathology slides

- Ensure the tissue section is flat. Carefully mount the tissue section into the center of the slide by carefully lowering the slide onto the tissue section. Mount each  $7\text{-}10\ \mu\text{m}$  thick tissue section within  $8\times 8\text{mm}$  of slide center (diagram below and template provided)
- Leave the slide with the tissue in the cryostat for  $\sim 5$  sec to allow the tissue section to refreeze and adhere to the slide. The tissue section should turn white once it refreezes and adheres.

AtlasXomics can analyze a  $5.2\times 5.2\ \text{mm}$  region of interest if tissue is placed within the blue region shown below:

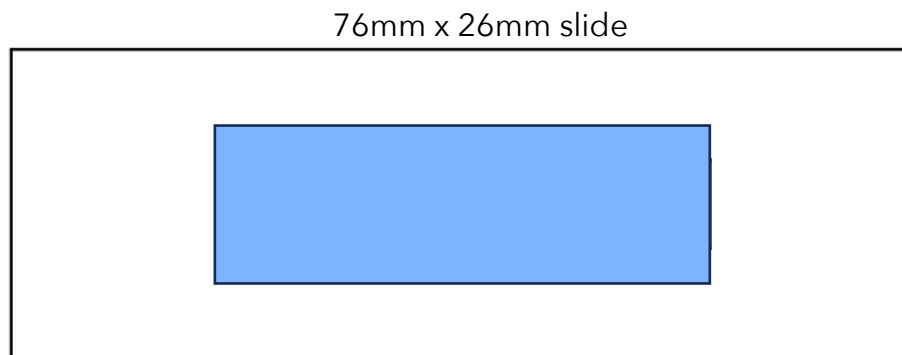


Figure 1

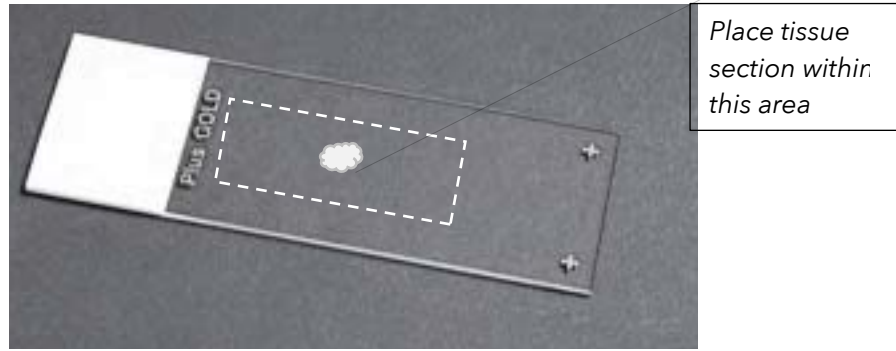


Figure 2. Schematic of tissue section on 1x3" slide

- **Label slide.** Annotate each mounted slide with a Sample ID (customer defined) using an Ethanol- and xylene-resistant marker ([Histo-Quill™](#) or similar<sup>1</sup>) or sharpie (not preferred).

**Storage**

- Place the slides in a shipping container (e.g. slide box holding 10-25 slides). Store slides at -80°C. DO NOT expose the slides to room temperature as the resulting condensation can cause tissue disintegration and degradation.
- When placing slides in slide box, ensure the edge labeled with Sample ID is closest to the top.

**Preparation for Shipping**

Fill in details in the shipping manifest. (See below, and file template provided).

*Full details should be recorded in the Shipping Manifest (Excel Template provided and referenced in Figure 3 and Figur).*

Box	Position	Block name	Sample ID (user defined)	Experimental Condition	Fixation	Species	Organ	Storage Conditions	Notes
1	1								
1	2								
1	3								
1	4								
1	5								
1	6								
1	7								
1	8								
1	9								

Figure 3

**Example completed Shipping Manifest:**

Box	Position	Block name	Sample ID (user defined)	Experimental Condition	Fixation	Species	Organ	Storage Conditions	Notes
1	1	NB_UK_lamda	lamda-1	Normal	Fresh Frozen (FF)	Mus musculus	Kidney	-80C	
1	2	NB_UK_lamda	lamda-2	Normal	Fresh Frozen (FF)	Mus musculus	Kidney	-80C	
1	3	NB_UK_lamda	lamda-3	Normal	Fresh Frozen (FF)	Mus musculus	Kidney	-80C	
1	4	NB_UK_lamda	lamda-4	Normal	Fresh Frozen (FF)	Mus musculus	Kidney	-80C	off center
1	5	NB_UK_lamda	lamda-5	Normal	Fresh Frozen (FF)	Mus musculus	Kidney	-80C	
1	6	NB_UK_27	27-1	Tumor	Fresh Frozen (FF)	Mus musculus	Kidney	-80C	
1	7	NB_UK_27	27-2	Tumor	Fresh Frozen (FF)	Mus musculus	Kidney	-80C	
1	8	NB_UK_27	27-3	Tumor	Fresh Frozen (FF)	Mus musculus	Kidney	-80C	
1	9	NB_UK_27	27-4	Tumor	Fresh Frozen (FF)	Mus musculus	Kidney	-80C	
1	10	NB_UK_29	29-1	Tumor	Fresh Frozen (FF)	Mus musculus	Kidney	-80C	for spatial

*Figure 4***Shipping:**

- Samples should be shipped overnight in dry ice. Ensure the dry ice fills the container, to keep samples frozen throughout transit and delivery time.
- Send tracking number and Shipping manifest to AtlasXomics.

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