STOmics

SAMPLE PREPARATION GUIDE FOR FRESH FROZEN SAMPLES ON Stereo-seq CHIPS OR Stereo-seq CHIP SLIDES

USER MANUAL

Manual Version: B

STUM_SP001

REVISION HISTORY

Manual Version:	A
Date:	Oct. 2024
Description:	Initial release.

Manual Version:	B
Date:	Aug. 2025
Description:	• Removed Chapter 3: CRYOSECTION PREPARATION. The instructions for cryosection preparation and tissue mounting have been integrated into the respective user manuals of each kit. • Added information and sample preparation requirements for the Stereo-seq Transcriptomics Large Chip Designs Solution.

Note: Please download the latest version of the manual and use it with the corresponding Stereo-seq Permeabilization kit.

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NOTE: Additional operation tips and guidance.

QUALITY CHECK POINT

CAUTION: Proceed with extra care; improper handling or carelessness may cause experimental failure or accidents.

CHAPTER 1
INTRODUCTION

1.1. Introduction

The STOmics Stereo-seq Transcriptomics Set is intended for generating a spatially-resolved 3' mRNA library from biological tissue sections requires a Stereo-seq Chip Slide with intact tissue sections as input. Prior to the Stereo-seq Transcriptomics workflow, optimal permeabilization time needs to be determined using the Stereo-seq Permeabilization Set for fresh frozen tissues. This guide provides general guidelines on how to properly perform tissue embedding, sectioning and mounting to better preserve the morphological quality of the tissue sections and the integrity of mRNA transcripts.

The Stereo-seq Chip Slides or Stereo-seq Chips prepared in this guideline are part of these products:

Stereo-seq Permeabilization Set for Chip-on-a-slide V1.1,
Cat. No.: 211SP11118 / 211SP11118-CG

Stereo-seq Transcriptomics Set for Chip-on-a-slide V1.3,
Cat. No.: 211ST13114 / 211ST13114-CG

Stereo-seq Transcriptomics Set for Chip-on-a-slide V1.3 (0.5cm * 0.5cm),
Cat. No.: 211ST13004 / 211ST13004-CG

Stereo-seq Permeabilization Set (1cm * 2cm) V1.1, Cat. No.: 111SP11124 / 111SP11124-CG

Stereo-seq Transcriptomics Set (1cm * 2cm) V1.3, Cat. No.: 111ST13122 / 111ST13122-CG

Stereo-seq Permeabilization Set (2cm * 2cm) V1.1, Cat. No.: 111SP11224 / 111SP11224-CG

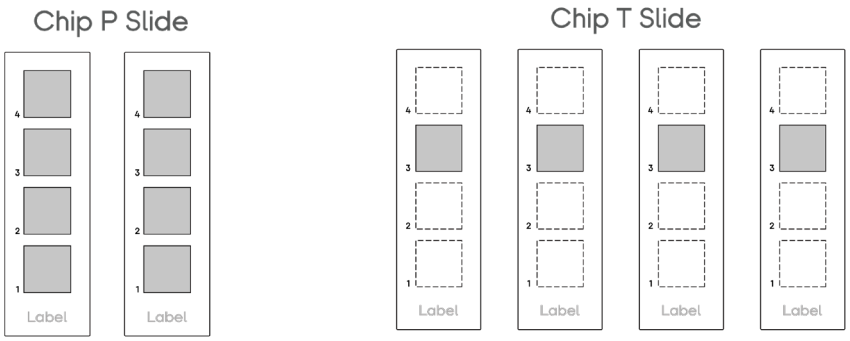
Stereo-seq Transcriptomics Set (2cm * 2cm) V1.3, Cat. No.: 111ST13221 / 111ST13221-CG

Stereo-seq Permeabilization Set (2cm * 3cm) V1.1, Cat. No.: 111SP11234 / 111SP11234-CG

Stereo-seq Transcriptomics Set (2cm * 3cm) V1.3, Cat. No.: 111ST13231 / 111ST13231-CG

Stereo-seq Chip P Slide and Chip T Slide

- Includes two Stereo-seq Chip P Slides containing **four** Chip P (1cm * 1cm) on each slide in each Stereo-seq Chip P Kit.
- Includes four Stereo-seq Chip T Slides containing **one** Chip T (1cm * 1cm) on each slide or **one** Chip T (0.5cm * 0.5cm) on each slide in each Stereo-seq Chip T Kit.
- Stereo-seq Chip P Slides and Stereo-seq Chip T Slides are differentiated by a laser-engraved label at the end of the slide.



Stereo-seq Chip P and Chip T (for Large Chip Designs Solution)

- Includes **four** Stereo-seq Chip P in each Stereo-seq Chip P Kit.
- Includes **two** Stereo-seq Chip T (1cm * 2cm) or **one** Stereo-seq Chip T (2cm * 2cm) or **one** Chip T (2cm * 3cm) in in each Stereo-seq Chip T Kit.

Stereo-seq Chip (Slide) Storage

Always store unused slides in their original slide container and keep them sealed with a sealable aluminum bag at 2°C ~ 8°C. Keep sealed with tape or a re-sealable bag. Always KEEP the desiccant within the bag.

CHAPTER 2
SAMPLE PREPARATION

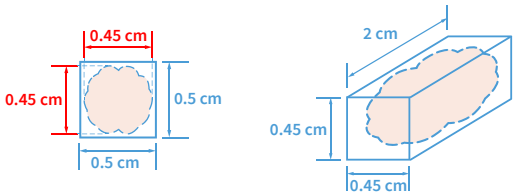
2.1. Sample Requirements for Fresh Frozen Tissue

To prevent RNA degradation, we recommend performing tissue embedding within **30 min** upon harvest.

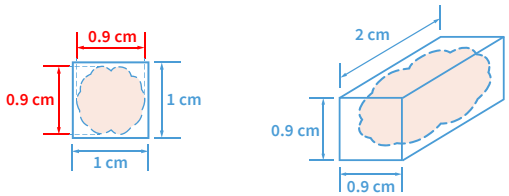
This manual also supports frozen sample embedding. However, if not properly stored, frozen samples may have fragmentation, deformation, or other suboptimal conditions. In addition to RNA integrity, tissue morphology should be carefully assessed before Stereo-seq experiments. It is crucial to ensure that RNA integrity and tissue morphology both meet the transcriptomics requirements before starting.

Based on chip specifications, there are three tissue sizes:

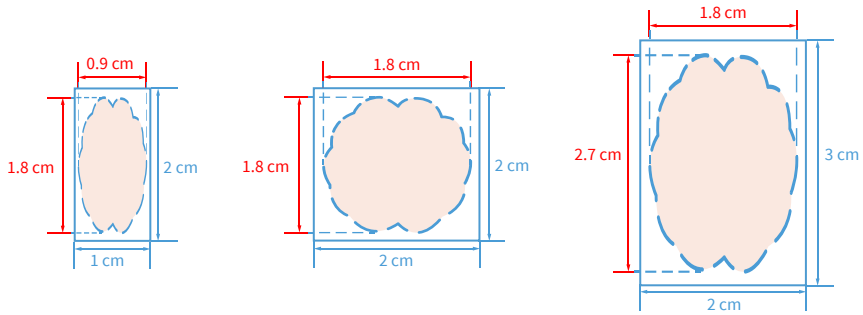
1. Stereo-seq chip (0.5 cm * 0.5 cm): The tissue size should not exceed **0.45 cm x 0.45 cm x 2 cm**, considering that the tissue section should not exceed 80% area coverage of the chip.



2. Stereo-seq chip (1 cm * 1 cm) : The tissue size should not exceed **0.9 cm x 0.9 cm x 2 cm**, considering that the tissue section should not exceed 80% area coverage of the chip.



3. Stereo-seq chip (1 cm * 2 cm): The tissue size should not exceed **0.9 cm x 1.8 cm x 0.7 cm**;
   Stereo-seq chip (2 cm * 2 cm): The tissue size should not exceed **1.8 cm x 1.8 cm x 0.7 cm**;
   Stereo-seq chip (2 cm * 3 cm): The tissue size should not exceed **1.8 cm x 2.7 cm x 0.7 cm**;
   considering that the tissue section should not exceed 80% area coverage of the chip.



Sample Types

For Stereo-seq Transcriptome FF Solution, Stereo-seq Transcriptomics mIF Solution, and Stereo-seq Large Chip Designs Solution: These product solutions are compatible with samples from all common animals, including but not limited to human, monkey, and mouse.

For Stereo-CITE Proteo-Transcriptomics Solution: This product solution is compatible with the simultaneous detection of multiple proteins and whole transcriptomes in both human and mouse samples.

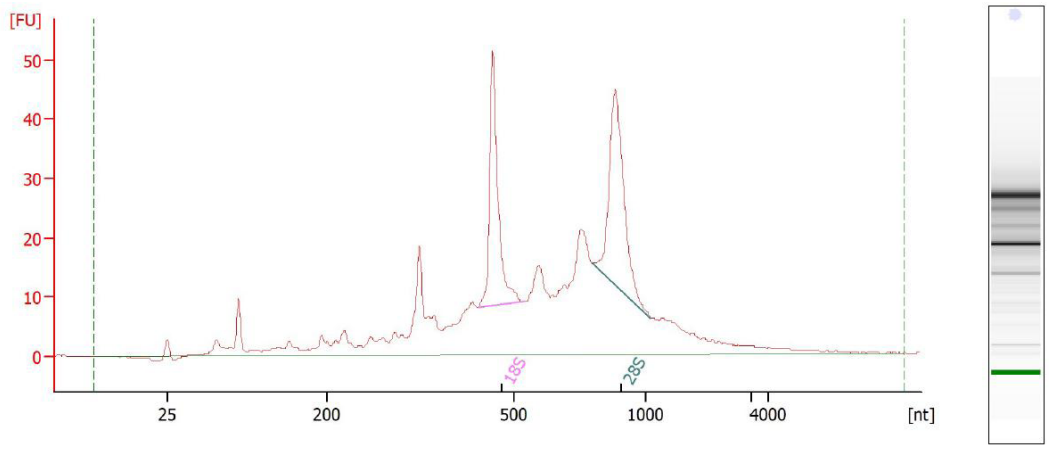
For details, refer to the STOmics Validated Tissue List at:

https://en.stomics.tech/resource/STOmicsTestedTissueList?lang=en#

FF Sample RNA Integrity Number (RIN) Value

It is recommended that you check the RNA quality (RIN value) of a tissue sample before proceeding to the Stereo-seq experiment. Collect 10 - 20 sections of 10 μm and store them at -20°C in a pre-cooled 1.5 mL EP tube for total RNA extraction. Refer to Figure 1 for the peak RNA RIN value in mouse brain tissue sections.

1. If you are using Stereo-seq Transcriptomics FF V1.3 Solutions, Stereo-seq Transcriptomics FF V1.3 for mIF Solutions, and Stereo-CITE Proteo-Transcriptomics V1.1 Solutions, it is recommended that you perform subsequent experiment operations only on tissue samples with **RIN value ≥4.**
2. If you are using Stereo-seq Transcriptomics Large Chip Designs, it is recommended that you perform subsequent experiments only on tissue samples with **RIN value ≥6.**
3. If you are using Stereo-seq Transcriptomics FF V1.2 Solutions, Stereo-seq Transcriptomics FF V1.2 for mIF Solutions, Stereo-CITE Proteo-Transcriptomics V1.0 Solutions, and Stereo-seq Transcriptomics Large-size Chip, it is recommended that you conduct subsequent experiments solely on tissue samples with **RIN value ≥7.**

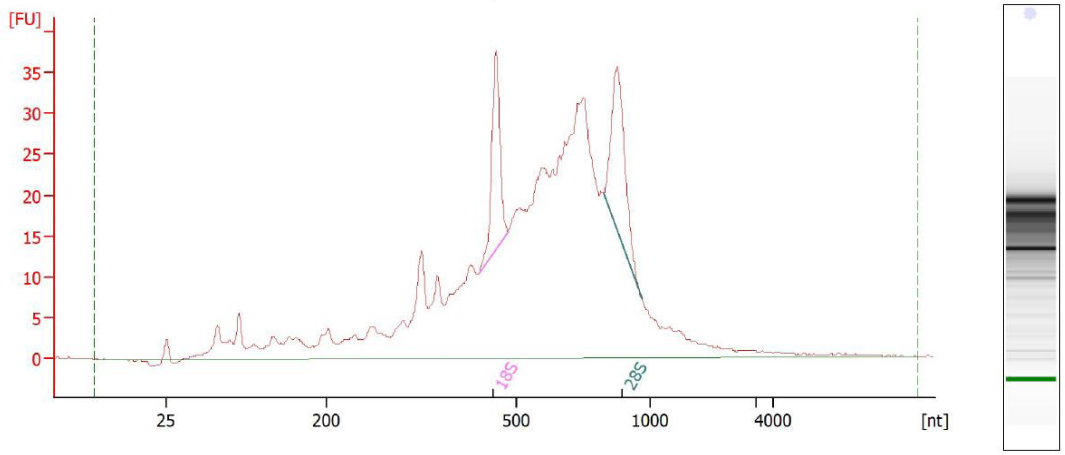


Overall Results for sample 5 : Sample 5

RNA Area:	558.0	RNA Integrity Number (RIN):	6.9 (B.02.10)
RNA Concentration:	18,318 pg/µl	Result Flagging Color:	
rRNA Ratio [28s / 18s]:	1.1	Result Flagging Label:	RIN: 6.90

Fragment table for sample 5 : Sample 5

Name	Start Size [nt]	End Size [nt]	Area	% of total Area
18S	441	535	59.6	10.7
28S	796	1,041	67.0	12.0



Overall Results for sample 7 : Sample 7

RNA Area:	672.5	RNA Integrity Number (RIN):	4.5 (B.02.10)
RNA Concentration:	22,077 pg/µl	Result Flagging Color:	
rRNA Ratio [28s / 18s]:	1.2	Result Flagging Label:	RIN: 4.50

Fragment table for sample 7 : Sample 7

Name	Start Size [nt]	End Size [nt]	Area	% of total Area
18S	441	485	31.6	4.7
28S	825	968	37.7	5.6

Figure 1. Example of RNA size distribution and RIN value measurement of tissue sections.

2.2. Sample Embedding for Fresh Frozen Tissue

Prepare the equipment and materials in advance. The following are the consumables or reagents required for embedding one sample. If multiple samples need to be embedded, use more consumables or reagents as necessary.

Materials		
Brand	**Description**	**Quantity**
-	Crushed ice in a container	1
-	Dry ice in a container	1
-	Aluminum Foil	1
-	Sealable Plastic Bag	1
BIOSHARP/Metal Coolbox/ BC032	Metal Block	1
-	Sterile Gauze	2
Corning	Corning® 35 mm TC-treated Culture Dish (353001)	1
Sakura/Base Molds/4583	O.C.T. Compound	1
Sakura/Base Molds/4132/4133 (or other brands of appropriately-sized embedding molds)	Stainless-steel Base Mold A	1
Sakura/Base Molds/4133/41565/4124 (or other brands of appropriately-sized embedding molds)	Stainless-steel Base Mold B (slightly larger than mold A)	1

Materials		
Brand	**Description**	**Quantity**
-	Blunt-end Forceps	1
-	Syringe	1
-	Spatula	1
-	Scissors	1
-	Steel Ruler	1

For a demonstration video of tissue embedding, refer to the link below or scan the QR code:

https://en.stomics.tech/resources/videos

a. Prepare these materials in advance:

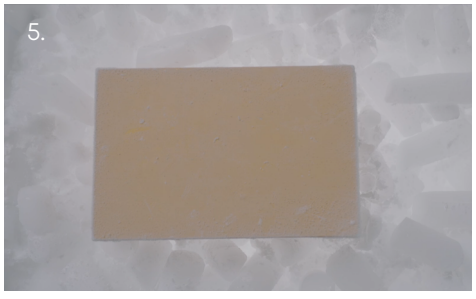
1. Pre-cool OCT on ice for **10 min** in advance.

2. Use **2** stainless-steel base molds slightly larger than the tissue of interest: mold A and mold B (slightly larger than mold A).

3. Add a few drops of pre-cooled OCT in mold A until it reaches approximately 2/3 of the mold and pre-cool on ice for **> 10 min** (remove introduced bubbles using a syringe).

4. In a petri dish filled with OCT, pre-cool the OCT on ice for **> 10 min** (remove introduced bubbles using a syringe).

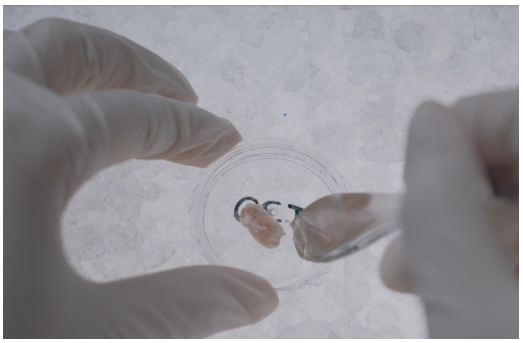
5. For this step, you will need a box of dry ice and a metal block that has a flat surface to support the stainless-steel base mold when placed on dry ice. The size of the metal block should be larger than the stainless-steel base mold. Place the metal block on dry ice and pre-cool for **> 5 min** with the flat surface facing up.



6. Place mold B on dry ice and pre-cool for **> 5 min** .

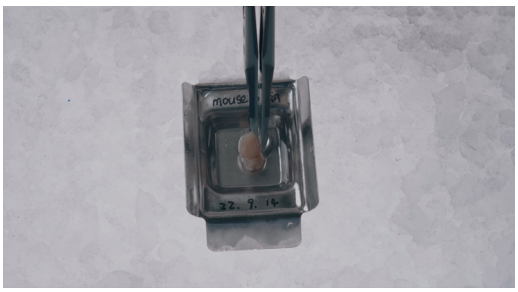
b. Upon harvesting within **30 min**, use sterile gauze or dust-free paper to absorb excess liquid on the tissue surface to prevent ice formation in later steps.

c. Place the tissue in pre-cooled OCT and wrap the tissue evenly with OCT using a spatula without introducing air bubbles.

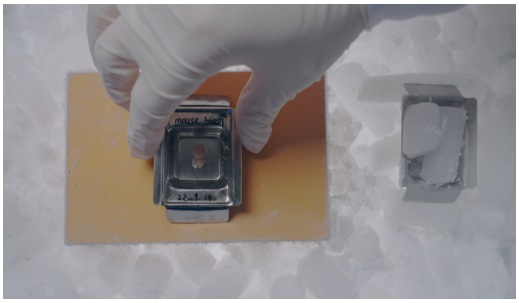


d. Remove any air bubbles using a syringe.

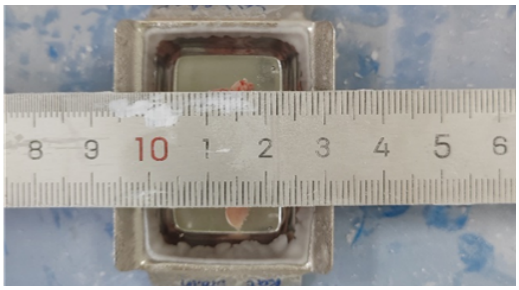
e. Orient the tissue such that the side intended for sectioning faces down, and then place it into mold A. Make sure the tissue is at the bottom of mold A, and then fill the mold with chilled OCT, without introducing bubbles, until the tissue is fully covered (remove any air bubbles using a syringe).

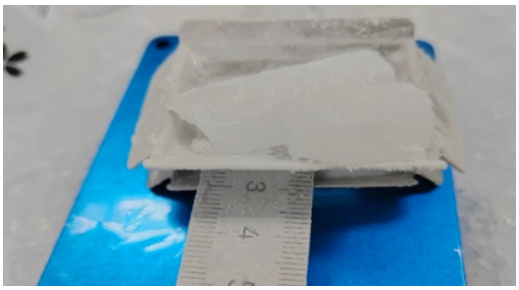


f. Place the tissue containing mold A onto the metal block that was placed on dry ice.



g. Place the pre-cooled steel ruler on the long and high side of mold A (to prevent the tissue from being compressed and deformed). Use mold B as a lid with the opening facing up, gently place it on top of mold A, and then place a few dry ice cubes on top of mold B. Make sure the two stainless-steel base molds can be completely covered with dry ice cubes.





h. After **5 min**, remove mold B and the steel ruler. Confirm that the OCT is completely frozen and has turned opaque; otherwise, repeat step f.

i. If the tissue block has solidified and turned opaque, grip the two edges of mold A and press the edges down to detach the tissue block from the mold.

j. Check the sectioning side of the tissue and determine if it is completely covered with OCT. If it is not completely covered, place the tissue block on the metal block, sectioning side up, add a few drops of OCT, and then wait until it solidifies and turns opaque.

k. Label the tissue block to mark the orientation of the tissue.

2.3. Sample Storage and Transportation

For storing, wrap the tissue block with aluminum foil and keep it in a properly labeled sealable plastic bag to prevent dehydration and damage, then store at -80°C. For transportation, ship samples **on dry ice** in accordance with local regulations.