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Overseas Shipping Guide (Tissues)

May 2023

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Standard Operating Protocol

Prepare samples according to stated guide

Complete Service Order Form (SOF) and send it to Mirxes

Prepare shipment package, air waybill and commercial invoice

Arrange for package pick up by your courier

Send the tracking number to Mirxes

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Sample Preparation (Non Stereo-seq)

For **assays other than Stereo-seq**, please send scrolls or sections sealed in an Eppendorf tube.

Blood can be sent using a regular blood tube.

Ensure you have ordered extraction in your purchase order

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Sample Preparation (Stereo-seq)

Sample Requirements

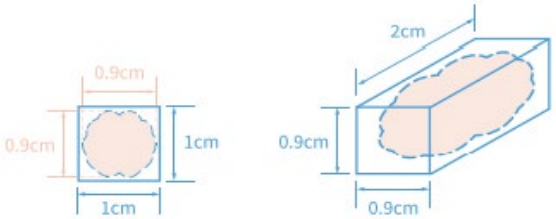
- The tissue size should be no more than 0.9 cm x 0.9 cm x 2cm, and appropriate cryomold size should be chosen based on the tissue size
- To avoid RNA degradation, we recommend performing tissue embedding within 30 min upon tissue harvesting
- Excess liquid on the tissue should be removed to avoid ice formation during embedding
- Air bubbles should be avoided when filling the OCT in the cryomold
- Please follow the steps in slide #5 for sample embedding
- It is highly recommended to submit the sample RNA Integrity Number (RIN) and a photo of H&E staining* (please refer slide #6 for more information)

Sample Preparation (Stereo-seq)

Sample Embedding – Apparatus to prepare in advance

Brand	Description	Quantity
-	Crushed ice in a box	1
-	Dry ice in a box	1
-	Aluminum foil	1
-	Sealable plastic bag	1
BIOSHARP	Metal Coolbox (Metal Block) - BC032	1
-	Sterile gauze	2
Corning	Corning® 35 mm TC-treated Culture Dish - 353001	1
Sakura	O.C.T	1
	Stainless-steel base mold - 4583/4162/7055	2
-	Blunt end forceps	1
-	Syringe	1
-	Spatula	1
-	Scissors	1

The tissue size should not exceed 0.9 cm x 0.9 cm x 2 cm, as the tissue section should not exceed 80% area coverage of the chip.



To avoid RNA degradation, we recommend performing tissue embedding within 30 min upon harvesting.

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Sample Preparation (Stereo-seq)

Sample Embedding – Apparatus to prepare in advance

1. A box of crushed ice and pre-cool OCT on ice for **10 min** in advance.

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

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

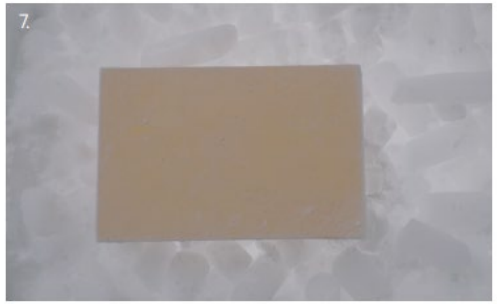
4. A petri dish filled with OCT and pre-cool it on ice for **> 10 min** (remove introduced air bubble using a syringe).

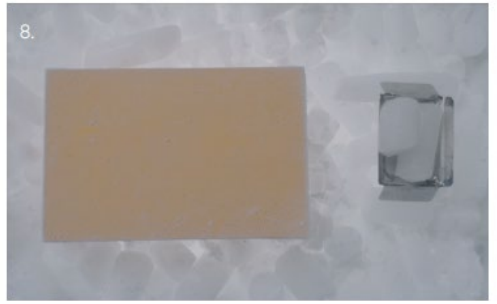
5. A box of dry ice.

6. 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.

7. Place the metal block on dry ice and pre-cool for **> 5 min** with the flat surface facing up.

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





Sample Preparation (Stereo-seq)

Sample Embedding – Step-by-step guide (1/2)

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

2. Place the tissue in pre-cooled OCT and wrap the tissue evenly with OCT using a spatula without introducing air bubbles (remove any air bubble using a syringe).

3. Orient the tissue to have the side intended to be sectioned facing downwards and then place into mold A. Make sure the tissue is at the bottom of mold A and fill the mold with chilled OCT without introducing bubbles until the tissue is fully covered.

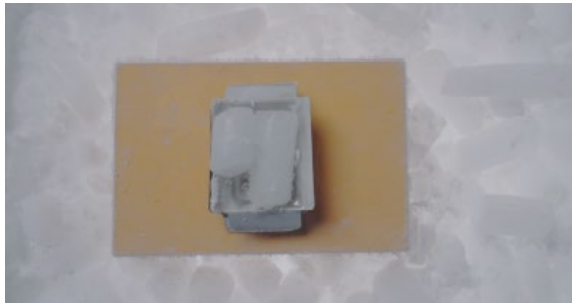
4. Place the tissue containing mold A onto the metal block that was placed on dry ice

For a demonstration video of tissue embedding, please refer to the link: https://drive.google.com/drive/folders/1O138SbfP8lKkYLaScnPkU3pOvwMf0NTW?usp=sharing

Sample Preparation (Stereo-seq)

Sample Embedding – Step-by-step guide (2/2)

5. Use mold B as a lid with opening facing up, place on top of mold A gently and then place a few dry ice cubes on top of mold B. Make sure the two stainless-steel base molds are nicely submerged in dry ice.



6. After **5 min**, remove mold B and check if the OCT is completely frozen and turns opaque, otherwise repeat step 5.

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

8. Check if the sectioning side of the tissue has been completely covered by OCT. If not, place the tissue block on the metal block, sectioning side facing up, add a few drops of the OCT and then wait till it solidifies and turns opaque. Label the tissue block to mark the orientation of the tissue. The OCT-embedded tissue block should be wrapped with aluminum foil, properly labelled, kept in a sealable plastic bag and stored at -80 °C if cryosectioning is not performed immediately.

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Sample Preparation (Stereo-seq)

RIN Determination and H&E Staining

- It is recommended to measure RIN **after** OCT embedding#
- Total RNA can be extracted from 10-20 slices of 10 µm-thick tissue sections to determine the RIN value (RIN≥7 is required)
- H&E staining of a tissue section **after** OCT embedding is required to check sample condition after embedding
- Make sure to coat the tissue with OCT immediately after trimming off sections for RIN or H&E staining
- The OCT-embedded tissue block should be wrapped with aluminum foil, properly labelled, kept in a sealable plastic bag and stored at -80 °C
- Please send us the Service Order Form (with RIN value fill in) together with a photo of H&E staining, and allow us to confirm the condition of the samples before arranging for sample shipment

 # If tissue size/amount is very limited, please check with your Mirxes contact if you can omit the RIN determination step

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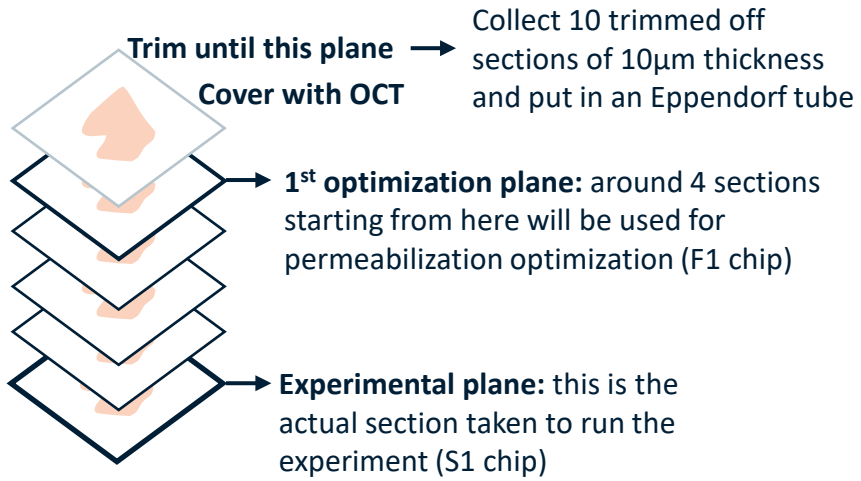
Sample Preparation (Stereo-seq)

Pre-Sample Submission

- The prepared OCT-embedded tissue block should be trimmed to the plane right before the 1st optimization plane
- Coat with OCT immediately to properly cover the tissue plane
- The OCT-embedded tissue block should be wrapped with aluminum foil, properly labelled, kept in a sealable plastic bag and stored at -80 °C
- Collect about 10 trimmed off tissue sections (10µm-thick for each section), put in an Eppendorf tube and send to us for RIN determination
- Send us the Service Order Form (with RIN value) together with a photo of H&E staining, and allow us to confirm the condition of the samples **before** arranging for sample shipment



Sample Preparation (Stereo-seq)

Sample preparation for multiple tissues on one chip

- Can multiple samples be mounted on one chip?

Yes, provided multiple samples can be embedded **on 1 cryomold** and the samples are of the same tissue type, the same size, and are embedded to the same plane. However, we recommend not to put more than 4 tissue samples on 1 cryomold to ensure adequate spacing between samples. It is equally important that the tissues are embedded in the center area of the cryomold to ensure a proper fit on the 1cm x 1cm chip.

- Can multiple diseased tissues of the same tissue type be mounted on one chip?

Yes, however, it is common knowledge that human tissue samples are highly heterogenous. The permeabilization conditions for different samples may vary a lot depending on tissue morphology, tissue architecture or the stage of the disease. We will work together with the customer to find out the most optimum permeabilization condition for multiple samples. This will be based on case-by-case discussions.

The Stereo-seq preparation guideline is adapted from BGI-Research

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Service Order Forms

Four types of service order forms (make sure you get the right one!)

1. Standard SOF: WGS, WES, RNA-Seq, WGMS
2. Stereo-seq SOF: For full Stereo-seq service only

Ensure you have ordered extraction!

Return the completed form to Mirxes by emailing a soft copy and attaching a printed copy in your package

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Shipment Preparation

- Ensure all tubes or plates are clearly labelled per your descriptions in the sample order form.
- Ensure all tubes are tightly capped and parafilm-sealed to prevent leakage of samples. For plates, we will recommend heat-sealing if available.
- Pack in sufficient dry ice or ice packs (we recommend at least 2 days worth).
- Ensure package is tightly sealed prior to shipping

Shipment Preparation – Non-human tissues

There will be an application process for an import permit of non-human tissues prior to shipping your samples to Singapore. All imported animal sources should not be on CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) list: https://cites.org/sites/default/files/eng/app/2023/E-Appendices-2023-05-04.pdf

Please ensure that the following documents are complete and submitted to your Mirxes contact.

1. Animal sources:
    a) Application for Import Permit of **Animal** Tissues for Research Purposes
    b) Veterinary health certificate certifying that the item was taken from animals which are healthy and clinically free from contagious disease, that these animals have not been exposed to any other matter of animal origin (if available)
2. Plant sources:
    a) Application for Import Permit of **Plant** Tissues for Research Purposes
    b) Phytosanitary certificate certifying that the plant conform with the current phytosanitary requirements of your country. This certificate is normally issued by the authority of agriculture of the country of origin of the plant

Please do not ship your tissues until your Mirxes contact confirms application is successful, else your shipment will be rejected at customs.

Air Waybill (AWB) Preparation

The AWB are important for customs, insurance, billing and proof of contract between the shipping parties.

Your information

Mirxes information

Mirxes Pte Ltd

8 Biomedical Grove #02-01 Neuros

Singapore 138665

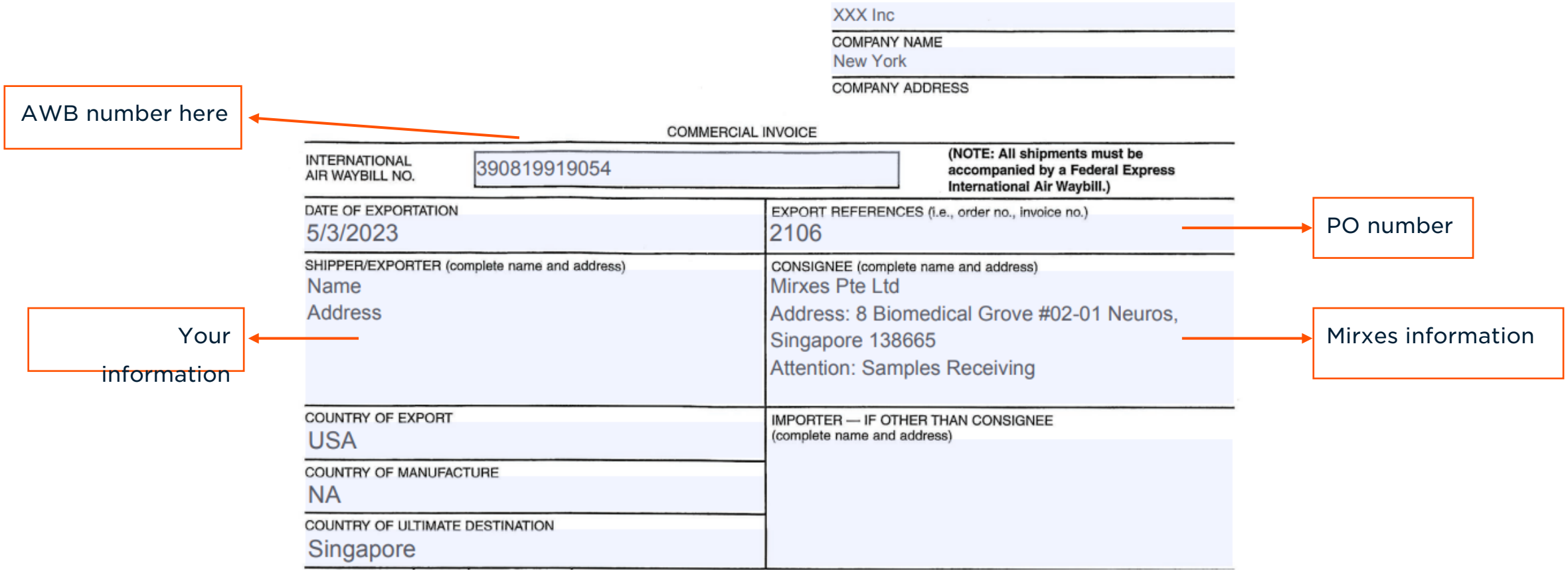
Attn: Samples Receiving

Input as “Non-hazardous, non-infectious research samples

Refer to http://www.fedex.com/hk_english/services/tools/intlairwaybill.html for a step-by-step guide. Besides FedEX, Mirxes also works with DHL and World Courier for shipments of biological materials.

Commercial Invoice Preparation

The commercial invoice is the primary document for customs clearance in international shipments.



Template from FedEX: http://www.fedex.com/downloads/shared/shipdocuments/blankforms/commercialinvoice.pdf

Commercial Invoice Preparation

Declare as stated here

(must be identical with your AWB)

MARKS/NOS.	NO. OF PKGS.	TYPE OF PACKAGING	FULL DESCRIPTION OF GOODS	QTY.	UNIT OF MEA-SURE	WEIGHT	UNIT VALUE	TOTAL VALUE
	1	Box	Non-hazardous, non-infectious research samples	1	EA	10kg	$50	$50

TOTAL NO. OF PKGS.	TOTAL WEIGHT	TOTAL INVOICE VALUE
1	10kg	$50

SEE REVERSE SIDE FOR HELP WITH THE ABOVE SECTION

FOR U.S. EXPORT ONLY: THESE COMMODITIES, TECHNOLOGY, OR SOFTWARE WERE EXPORTED FROM THE UNITED STATES IN ACCORDANCE WITH THE EXPORT ADMINISTRATION REGULATIONS. DIVERSION CONTRARY TO UNITED STATES LAW IS PROHIBITED.

I DECLARE ALL THE INFORMATION CONTAINED IN THIS INVOICE TO BE TRUE AND CORRECT.

SIGNATURE OF SHIPPER/EXPORTER (Type name and title and sign.) DATE

XXX 5/3/2023

M-1054 REV 2/00 LOGOS 116547 WCS

Check one
- [x] F.O.B.
- [ ] C & F
- [ ] C.I.F.

Declare any value between $1 - $50

(must be identical with your AWB)

Remember to check FOB

Template from FedEX: http://www.fedex.com/downloads/shared/shipdocuments/blankforms/commercialinvoice.pdf

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Ship your samples

- Shipments are typically received on weekdays from 9am to 6pm barring public holidays in Singapore
- Recommended to ship on Monday (to reach Thurs-Fri) or Friday (to reach Mon-Tues)
- Mirxes can accept urgent shipments on weekends if required
- Please refer here for the public holidays in Singapore to avoid
- At this point of time, you should have a technical specialist communicating with you on the process and he will be able to assist you with your questions