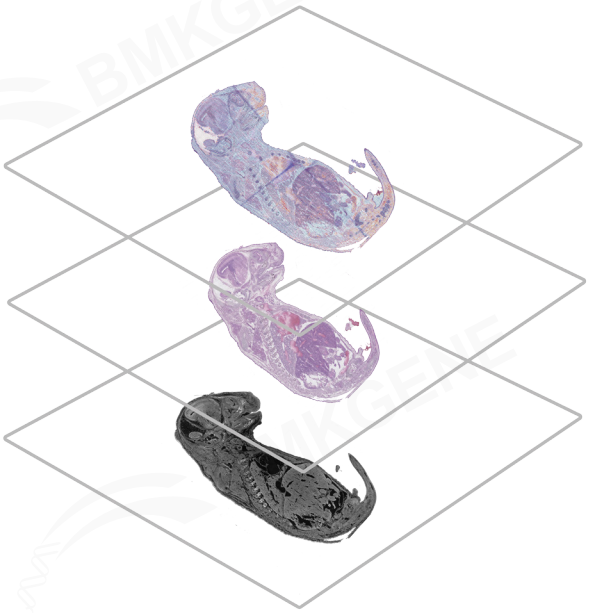
BMKGENE

Sample Preparation Guidance for Spatial Transcriptomics

(For Animal Samples)



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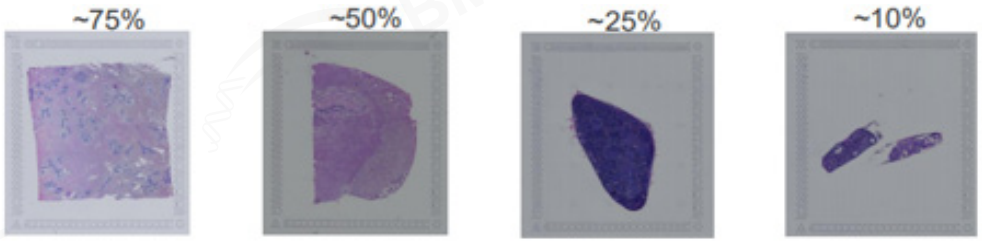
Content

1. Sample Requirements

For 10x Spatial Transcriptomics, tissue samples should not exceed 6.5 x 6.5 mm^2 in length and width, while for BMKMANU S3000 Spatial Transcriptomics, tissue samples should not exceed 6.8 x 6.8 mm^2 in length and width. The thickness should be greater than 1.5 mm.

For tissues with a tissue section coverage of less than 25%, we recommend embedding multiple samples into one OCT embedding block with minimal tissue spacing, but no overlapping. Multiple tissues should be kept on the same horizontal level to fully utilize the capture area.



Note 1 -Animal Tissue:

- If sample quantity permits, it is recommended to prepare at least two embedding blocks for each animal sample.

- One block should be used for RNA quality control (slice quality control standards are outlined in section 3), slice selection, and for applying both the permeabilization and expression chips. The second block should be used as a backup to avoid project delays in case of abnormal sample conditions or other unforeseen circumstances requiring resampling.

2. Sample Preparation and Transportation

2.1 Pre-Sampling Preparation

(1) All instruments and environments should be sterilized and disinfected, and all instruments (such as scissors, forceps, etc.) should be pre-cooled on ice.

(2) Place a layer of ice on a medical tray, cover it with aluminum foil, and then lay several layers of sterile gauze. Place the individual on the sterile gauze for sampling, and keep the entire sampling process at low temperature to delay nucleic acid degradation. (For animal tissues, it is recommended to perform dissection sampling on live or recently deceased individuals).

(3) If subsequent embedding experiments cannot be performed immediately after sampling animal tissues, the sample can be temporarily stored in tissue storage solution (such as Miltenyi, Cat# 130 -100-008) or DPBS or physiological saline and placed in a 4°C refrigerator. The storage time should not exceed 5 hours to avoid RNA degradation and unsatisfactory RIN value quality control.

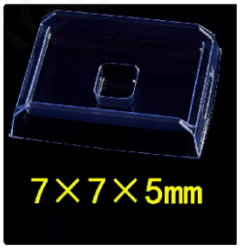
(4) Use the designated brand of OCT (Sakura-4583 or Leica-FSC 22) for embedding, and pre-cool the OCT on ice for 30 minutes before use.

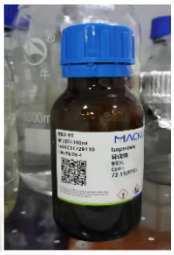
2.2 Sampling (Animal Sample)

(1) Fresh tissues should be obtained and non-research-required tissue types such as connective and adipose tissues should be immediately removed. For tumor tissue collection, the tumor and normal tissues should be accurately distinguished as much as possible, and the surrounding normal tissues should be cleanly excised from the tumor tissue (the surrounding tumor tissues should also be cleanly excised from the normal tissue).

(2) Quickly rinse off the remaining blood on the tissue surface using pre-cooled PBS solution (RNase -free) or physiological saline, and then absorb the surface liquid with sterile gauze.

(3) If the tissue volume is large, it needs to be cut into small pieces with a length and width less than 6.5*6.5 mm^2 (10x Genomics) / 6.8*6.8 mm^2 (BMKMANU S3000) and embedded in an extra small embedding cassette with a size of 7*7*5 mm. The obtained tissue should be immediately subjected to subsequent embedding experiments.



Recommended OCT embedding medium (Sakura-4583)
Minimum size of embedding cassette
Isopentane purity: 95% (CAS: 78-78-4) (If not obtainable, dry ice pellets can be used as a substitute.)

Note: Reagents for tissue freezing and embedding are not provided by BMKGENE. Customers need to prepare them.

2.3 Freezing and Embedding of Animal Tissue

Two embedding protocols are provided below: dry ice embedding and isopentane + liquid nitrogen embedding. Customers can choose the appropriate method according to their experimental conditions. Dry ice embedding is suitable for a wider range of tissue types, is the most commonly used embedding method, and can also obtain good slicing results. Isopentane + liquid nitrogen embedding can rapidly cool the tissue and protect cell integrity, but the operation is more complex, and isopentane poses a certain health hazard to humans. For smaller or lighter samples such as biopsy samples, tissues prone to ice crystal formation such as brain tissue, or edematous pathological tissues, it is recommended to use the liquid nitrogen + isopentane method for freezing and embedding.

Protocol 1: Dry Ice Embedding (Commonly Used)

Isopentane is not required. Embed directly with dry ice pellets.

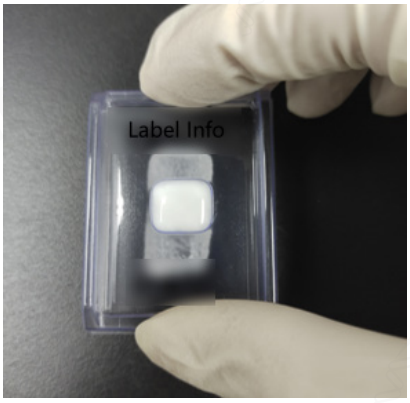
(1) Place fresh tissue in a culture dish and use clean gauze or paper towels to dry the blood around the tissue as much as possible, making the tissue surface as dry as possible.

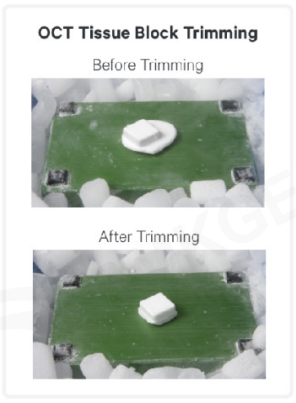
(2) Label the embedding cassette (sample name, sample orientation, embedding date, etc.). It is important to label the embedding cassette before freezing, as it will be difficult to label the information once the cassette is frozen.

(3) Transfer the embedding cassette to ice and inject pre-cooled OCT (~ 1/4) into it to avoid bubbles.

(4) Pre-cool the forceps on ice, place the tissue in the OCT, adjust the orientation, cover any exposed tissue surface with OCT, and confirm that there are no bubbles, especially near the tissue. If there are bubbles, use a pipette to remove them to avoid affecting the morphology and structure of the tissue slices. (This step requires taking a photo record, as OCT will turn white after freezing, making it difficult to determine the orientation of the tissue.)

(5) Gently press the embedding block containing tissue and OCT onto dry ice pellets (as shown on the left in the figure below) to ensure that it is level (otherwise, it will affect the orientation of the tissue). At the same time, it can ensure that the contact area between the dry ice pellets and the embedding block is maximized to achieve rapid cooling. Continue pressing until the embedding block is completely frozen and turns white (as shown on the right in the figure below).





Dry ice pellet quick freezing of embedding block Embedding block after OCT freezing.

(6) Wrap the OCT-embedded tissue block and embedding cassette in aluminum foil, label the sample, and store it directly in a sealed container at -80℃. Use dry ice for transportation.

Protocol 2: Isopentane + Liquid Nitrogen Embedding

For tissues prone to ice crystal formation such as brain tissue, or edematous pathological tissues, this embedding protocol is recommended.

(1) Fill two-thirds of a metal beaker with isopentane (enough to completely submerge the embedding cassette /tissue) and then place it in a Dewar flask containing liquid nitrogen (liquid nitrogen and isopentane should maintain the same level) for full contact and incubate for 15 minutes.

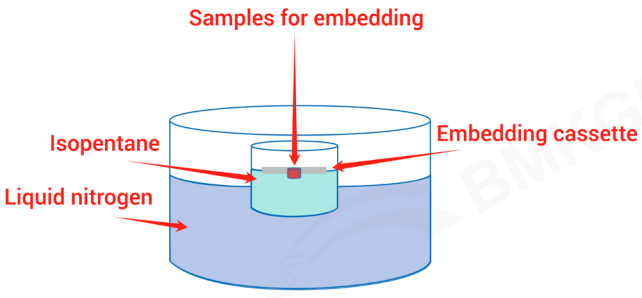
(2) Place fresh tissue in a culture dish and use clean gauze or paper towels to dry the blood around the tissue as much as possible, making the tissue surface as dry as possible.

(3) Label the embedding cassette (sample name, sample orientation, embedding date, etc.). It is important to label the embedding cassette before freezing, as it will be difficult to label the information once the cassette is frozen.

(4) Transfer the embedding cassette to ice and inject pre-cooled OCT (~ 1/4) into it to avoid bubbles.

(5) Pre-cool the forceps on ice, place the tissue in the OCT, adjust the orientation, cover any exposed tissue surface with OCT, and confirm that there are no bubbles, especially near the tissue. If there are bubbles, use a pipette to remove them to avoid affecting the morphology and structure of the tissue slices. (This step requires taking a photo record, as OCT will turn white after freezing, making it difficult to determine the orientation of the tissue.)

(6) Use forceps to place the embedding cassette in isopentane, but do not submerge the isopentane into the embedding cassette until the tissue is frozen. The freezing time can vary depending on the type and size of the tissue (As shown in the figure below).

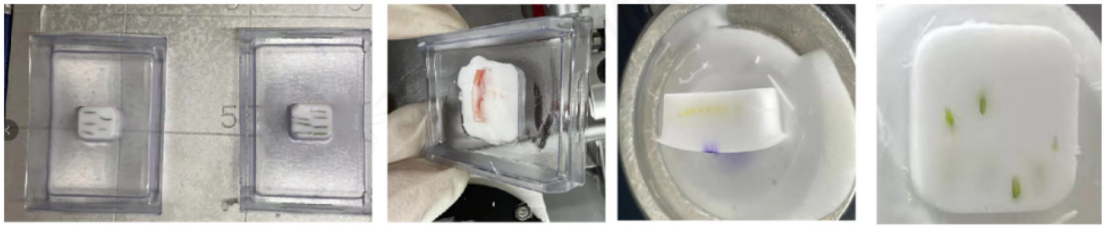


(7) Wrap the OCT-embedded tissue block and embedding cassette in aluminum foil, label the sample, and store it directly in a sealed container at -80℃. Use dry ice for transportation.

2.4 Sample Transportation

After embedding, wrap the tissue in aluminum foil, seal it in a sealed bag, and place it in a sample box for long-term storage at liquid nitrogen or -80℃ freezer. Alternatively, place it on dry ice for frozen transportation. For dry ice shipment, it is recommended to use approximately 5 kg per day.

Examples of Non-standard Sample Submission:



A B C D

A. The tissue is placed near the bottom during embedding, and the tissue is not wrapped in OCT, resulting in RNA degradation and failed quality inspection.
B. The tissue is exposed to air, resulting in sample degradation. The tissue is not supported by OCT on both sides, which may damage the sample structure during sectioning.
C. The tissue is too small to meet the minimum requirement of 25% coverage for expression analysis.
D. Multiple samples are embedded in the same block, but not in the same plane, and each sample tissue is too small and has limited coverage area, which may result in lower effective data.

3. Section Quality Control Standards

Tissue morphology inspection: Perform H&E staining on the sections to observe whether the target area is obtained and whether the integrity of tissue morphology is within an acceptable range (e.g. whether there are large cracks, obvious bubbles, wrinkles, etc.).

RNA integrity inspection: Take 10-15 sections and use Agilent 2100 (or equivalent) to inspect RNA integrity. It is required that RNA RIN ≥ 6, which indicates that the sample integrity meets the experimental requirements and can proceed to subsequent experiments. The specific section quality inspection quantity is as follows:

(1) 10 μm section thickness, tissue area accounts for 1/5-1/4 of the embedding block surface area, and the quality inspection quantity is ~20 sections (left figure).

(2) 10 μm section thickness, tissue area accounts for > 1/3 of the embedding block surface area, and the quality inspection quantity is ~15 sections (right figure).