Preparing Fresh Frozen Tissue Blocks for the RNAscope™ and BaseScope™ Assays

This protocol is intended as a guide only, for full experimental details please read the product user manual.

Introduction

This Technical Note provides guidelines to prepare OCT-embedded fresh frozen tissue for the RNAscope™ 2.5 and BaseScope™ Assays. For pretreatment conditions, refer to the *Sample Preparation Technical Note for Fresh Frozen Tissue Using RNAscope 2.5 Chromogenic Assay* (Doc. No. TN-320536). Wear appropriate protective eyewear, clothing, and gloves. For the latest services and support information, contact our Technical Services.

Part 1: Frozen Tissue Processing

1. Harvest animal tissues within 5 MIN of sacrifice. Cut dissected tissues into pieces ≤ 5 mm in thickness, and keep on ice.
2. Select an appropriately sized cryomold to leave at least 5 mm of space between the tissue and the block edge.
3. Place at least 1 lb of dry ice in a styrofoam box.
4. Create a flat surface in the dry ice to stabilize the cryomold. For example, use a metal block and let the block temperature equilibrate to that of dry ice for 10 MIN. Alternatively, pour isopentane into the container until the level of liquid is slightly above the layer of dry ice. Let the bubbling subside before use.
5. Place tissues on a paper towel to remove excess liquid.
6. Add one drop of OCT to the bottom of the mold, and place the tissue in the desired orientation on top. Tissue will be sectioned starting from the bottom of the mold.
7. Carefully add more OCT until the tissue is completely covered and the cryomold is filled. Remove any bubbles that form near the tissue with a forceps.
8. Place the mold on the flat surface you created in the dry ice, or in cooled isopentane.
9. Let the OCT solidify completely. Keep the mold on dry ice for another 5 MIN. Wrap frozen blocks with labeled foil, and store at –70°C. You may also proceed directly to frozen tissue sectioning.

Part 2: Frozen Tissue Sectioning

1. Set cryostat temperature to –15°C to –20°C. Temperature is tissue dependent.
2. Remove and discard the microtome blade. Wipe down the knife holder and anti-roll plate with 100% ethanol.
3. Install a new microtome blade.
4. Place a slide box on dry ice, or inside the cryostat.
5. Set the cutting thickness to 10 µm (+/– 5 µm).
6. Transfer the frozen tissue from the freezer to the cryostat, transporting on dry ice if necessary.
7. Remove the tissue block from the plastic mold, and equilibrate it to the temperature of the cryostat 15–30 MIN.
8. Mount the tissue block onto the specimen holder using OCT. Allow the OCT to completely solidify.
9. Section the block, and mount the sections onto room temperature SuperFrost® Plus slides. Immediately place each slide into the slide box.

IMPORTANT! You must use SuperFrost® Slides (Fisher Scientific, Cat # 12-550-15). Do not allow the slides to come to room temperature. Keep cold at all times.

Allow the slides to dry for a minimum of 1 HR in the slide box before proceeding to the RNAscope or BaseScope™ assay.

IMPORTANT! Do not dry slides at room temperature.

NOTE: You may store the slides at –70°C for up to three months. Use the slides within three months to ensure RNA quality.

Obtaining Support

For the latest services and support information, contact our Technical Services.

At the website, you can:

- Access telephone and fax numbers to contact Technical Support and Sales
- Search through FAQs
- Submit a question directly to Technical Services

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