

# Preparing Fixed Frozen Tissue for the RNAscope® Fluorescent Multiplex Assay

## Introduction

This Technical Note is for customers who wish to run the RNAscope® Fluorescent Multiplex Assay (Cat. No. 320850) with fixed frozen tissues. The required pretreat reagents are RNAscope® Target Retrieval and RNAscope®

Protease III (available in available in RNAscope Universal Pretreatment Kit, Cat. No 322380). Refer to the user Safety Data Sheet (SDS) available on the ACD website.

## Workflow

### Part 1: Prepare the Tissue Sections

#### Fix Sample

1. If needed, perfuse tissue with freshly prepared 4% paraformaldehyde (PFA) in 1X PBS, or go directly to step 2.
2. Dissect tissue and place in freshly prepared 4% PFA for **24 HRS** at **4°C**.

#### Freeze Tissue

1. Immerse the tissue in 10% sucrose in 1X PBS at **4°C** until the tissue sinks to the bottom of the container (approximately **18 HRS** for brain tissue).
2. Repeat this step with 20% sucrose in 1X PBS, followed by 30% sucrose in 1X PBS, each time allowing the tissue to sink to the bottom of the container.
3. Freeze the tissue in the Optimal Cutting Temperature (OCT) embedding media with dry ice or liquid nitrogen and store it in an airtight container at **-80°C**.

#### Prepare Sections

1. Before tissue sectioning, equilibrate the tissue blocks at **-20°C** for at least **1 HR** in a cryostat.
2. Section the blocks by cutting 7–15 µm sections. Mount the sections on SuperFrost® Plus slides (Fisher Scientific # 12-550-15).

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**IMPORTANT!** Use only SuperFrost® Plus slides. Other slide types may result in tissue loss.

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3. Air dry the slides for **20 MIN** at **-20°C**, or if slides are not used immediately store the sections at **-80°C** for **< 3 MONTHS**.
4. Wash the slides with 200 mL 1X PBS in a Tissue-Tek® slide rack for **5 MIN** while moving the rack up and down to remove OCT.

### Part 2: Tissue Pretreatment

#### Prepare Materials

1. Bring HybEZ™ Oven to **40°C**.
2. Place a wet humidifying paper in the Humidity Control Tray, leaving the HybEZ™ Slide Rack on bench. Re-insert the covered tray into the oven and close the oven door. The tray should be pre-warmed for at least **20 MIN** before use.
3. Prepare 700 mL fresh 1X Target Retrieval in a beaker. Cover with foil, bring to a mild boil, and maintain uniform boiling at **99–100°C**. Do not boil more than **30 MIN** before use.

#### Apply RNAscope® Target Retrieval

1. With a pair of forceps *very slowly* submerge a slide rack containing the slides into boiling 1X Target Retrieval solution for **5 MIN**.

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**Note:** Depending on tissue type, boiling time may need to be adjusted.

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2. *Immediately* transfer the hot slide rack to a staining dish containing distilled water.
3. Wash slides in distilled water by moving the rack up and down 3–5 times. Repeat with fresh distilled water.
4. Wash slides in fresh 100% EtOH by moving the rack up and down 3–5 times. Air dry.
3. Place slides in a Tissue-Tek® Slide Rack submerged in distilled water.
4. Wash the slides by moving the rack up and down 3–5 times. Repeat with fresh distilled water.

#### **Create Barrier**

1. Draw 2–4 times around tissue using the Immedge™ hydrophobic barrier pen. Let the barrier dry completely ~**1 MIN** or **OVERNIGHT** at **RT**.

#### **Apply RNAscope® Protease III**

1. Place the slides on the HybEZ™ Slide Rack, and add 2–4 drops Protease III to each section. Use enough solution to completely cover the sections.
2. Place the HybEZ™ Slide Rack in the pre-warmed HybEZ™ Humidity Control Tray. Seal tray and insert back into the HybEZ™ Oven. Incubate for **30 MIN** at **40°C**.

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**IMPORTANT!** Proceed to the RNAscope® protocol using the *RNAscope® Fluorescent Multiplex Kit User Manual Part 2* (Catalog No. 320293) available at <http://www.acdbio.com/technical-support/user-manuals>.

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#### **Obtaining Support**

For the latest services and support information, go to:  
<https://acdbio.com/technical-support/support-overview>.

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 Support.

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