Sample Preparation Technical Note for Fixed Frozen Tissue Using RNAscope® 2.5 Chromogenic Assay (Single-plex and Duplex)

Introduction

This Technical Note provides guidelines for preparing and pretreating fixed frozen tissue using an RNAscope® 2.5 Chromogenic Detection Kit (Cat. No 322310, 322360, or 322500). The required pretreatment reagents are RNAscope® Hydrogen Peroxide, RNAscope® Target Retrieval and RNAscope® Protease Plus (available in RNAscope Universal Pretreatment Kit, Cat. No 322380). Refer to the Safety Data Sheet (SDS), available on the ACD website. Materials required, but not provided by ACD, include sucrose, 1X PBS, OCT media, 100% EtOH, Superfrost® Plus slides (Fisher), and 4% paraformaldehyde (4% PFA).

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).

Note: The time needed for the tissue to sink varies with tissue type and size.

---

2. Immerse the tissue in 20% sucrose in 1X PBS at **4°C** until the tissue sinks to the bottom of the container.
3. Immerse the tissue in 30% sucrose in 1X PBS at **4°C** until the tissue sinks to the bottom of the container.
4. Freeze the tissue in OCT (Optimal Cutting Temperature) embedding media or TFM (Tissue Freezing Media) with crushed dry ice or iso-pentane or liquid nitrogen.
5. Store tissue blocks in an airtight container at **-80°C**.

Prepare Sections

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

IMPORTANT! Only use SuperFrost® Plus slides. Other slide types may result in tissue loss.

---

3. Air dry the slides for **2 HR** at **-20°C** and overnight at **-80°C**. If all the slides are not used immediately, store them at **-80°C** for up to **3 MONTHS**.
4. On the day of performing the RNAscope® assay, 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.
5. Bake the slides for **30 MIN** at **60°C**.
6. Post-fix the slides by immersing them prechilled 10% NBF or 4% PFA in 1X PBS for **15 MIN** at **4°C**.

Dehydrate the tissue

1. Prepare 200mL 50% EtOH, 200 mL 70% EtOH, and 400 mL of 100% EtOH.
2. Remove the slides from the 10% NBF or 4% PFA, and immerse them in 50% EtOH for **5 MIN** at **RT**.

3. Remove the slides from 50% EtOH, and immerse them in 70% EtOH for **5 MIN** at **RT**.
4. Remove the slides from 70% EtOH, and immerse them in 100% EtOH for **5 MIN** at **RT**.
5. Remove the slides from 70% EtOH, and immerse them in 100% EtOH for **5 MIN** at **RT**.

Dry the slides

1. Remove slides from 100% EtOH, and let them air dry for **5 MIN** at **RT**.

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 ACD EZ-Batch™ 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® Hydrogen Peroxide

1. Add 2–4 drops Hydrogen Peroxide to each section for **10 MIN** at **ROOM TEMPERATURE (RT)**. Use enough solution to completely cover the sections. Then rinse once in distilled water.

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 and keep them in the solution for **5 MIN** only. Please monitor the temperature closely and make sure it stays at 98-102**°C**.

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

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. Rinse slides in fresh 100% EtOH by moving the slides up and down 3–5 times. Air dry.

Create Barrier

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

Apply RNAscope® Protease Plus

1. Place the slides in the ACD EZ-Batch™ Slide Tray (slide locking tray) and add 2–4 drops Protease Plus to each section. Use enough solution to completely cover the sections.
2. Place the ACD EZ-Batch™ Slide Tray in the pre-warmed HybEZ™ Humidity Control Tray. Seal tray and insert back into the HybEZ™ Oven. Incubate for **30 MIN** at **40°C**.
3. Wash slides in the clear EZ-Batch Wash Tray by submerging the slides in distilled water.
4. Wash the slides with slight agitation. Repeat with fresh distilled water.

IMPORTANT! Proceed to the RNAscope® protocol using the appropriate Part 2 Detection User Manual* available http://www.acdbio.com/technical-support/user-manuals

**RNAscope® 2.5 HD Detection Kit-Brown User Manual, Part2 (Doc. No.322300_USM); RNAscope® 2.5 HD Detection Kit-Red User Manual, Part 2 (Doc. No. 322350_USM); RNAscope Duplex Detection Kit- Chromogenic User Manual (Doc. No.322500_USM)*

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

Headquarters
7707 Gateway Blvd Suite 200, Newark, CA 94545 Phone 1-510-576-8800 Toll Free 1-877-576-3636
For support, email **support.acd@bio-techne.com**
www.acdbio.com

For Research Use Only. Not for diagnostic use.

NOTICE TO PURCHASER: PLEASE REFER TO THE RNASCOPE® 2.5 ASSAY- USER MANUAL FOR LIMITED USE LABEL LICENSE OR DISCLAIMER INFORMATION. Advanced Cell Diagnostics, Inc. reserves the right to change its products and services at any time to incorporate technological developments. This manual is subject to change without notice. Although this manual has been prepared with every precaution to ensure accuracy, Advanced Cell Diagnostics, Inc. assumes no liability for any errors, omissions, or for any damages resulting from the use of this information.