ADVANCED CELL DIAGNOSTICS

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 320700). The required pretreat 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. http://www.acdbio.com/technical-support/user-manuals

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).

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

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™ or 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 for **5 MIN**.

   Note: Depending on 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. Wash slides in fresh 100% EtOH by moving the rack up and down 3–5 times. Air dry.

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 Plus

1. Place the slides on the HybEZ™ or ACD EZ-Batch™ Slide Rack, and add 2–4 drops Protease Plus to each section. Use enough solution to completely cover the sections.
2. Place the HybEZ™ or ACD EZ-Batch™ 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**. Place slides in a ACD EZ-Batch™ Slide Rack submerged in distilled water.
3. Wash the slides by moving the rack up and down 3–5 times. 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 2-Plex Detection Kit- Chromogenic User Manual (Doc. No.320791_USM)*

Obtaining Support

For the latest services and support information, go to: http://www.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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