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

Introduction

This Technical Note is intended for use of Fresh Frozen Tissue with the RNAscope® Chromogenic assays. For single and duplex chromogenic assays, the required pretreatment reagents are RNAscope® Hydrogen Peroxide and RNAscope® Protease IV. For Part 2 of the detection assay procedures, refer to the specific RNAscope® Chromogenic Detection assay manual available on the ACD website. See the Safety Data Sheet (SDS) also available on the ACD website **http://www.acdbio.com/technical-support/user-manuals**.

Workflow

Part 1: Prepare the Tissue Sections

Section Preparation

1. Cryosection the tissue to 10–20µm thickness and place onto SuperFrost Plus slides. Store slides at room temperature.
2. Keep the sections at **–20°C** to dry for 1 hour.
3. Store the sections at **–80°C**.
4. Sections may be stored up to 3 months at **–80°C**.

NOTES: Do not process the slides with any fixative (alcohol or formaldehyde) before this step.

The slides can be shipped on dry ice.

Sample Fixation

1. Pre-chill 200 mL of 10% neutral buffered formalin (NBF) or 4% paraformaldehyde (PFA) in 1X PBS to **4°C**.
2. Remove fresh frozen tissue slides from **–80°C**. Immediately immerse the slides in the pre-chilled 10% NBF or 4% PFA.
3. Incubate the slides for **15 MIN** at **4°C**.

Dehydrate the Tissue

1. Prepare 200 mL 50% EtOH, 200 mL 70% EtOH, and 400 mL 100% EtOH.
2. Remove the slides from NBF or 4% paraformaldehyde. Immerse in 50% EtOH. Incubate for **5 MIN** at **ROOM TEMPERATURE (RT)**.
3. Remove the slides from 50% EtOH. Immerse in 70% EtOH. Incubate for **5 MIN** at **RT**.
4. Remove the slides from 70% EtOH. Immerse in 100% EtOH. Incubate for **5 MIN** at **RT**.
5. Remove the slides from 100% EtOH. Immerse in fresh 100% EtOH. Incubate for **5 MIN** at **RT**.
6. Store the slides in 100% EtOH at **–20°C** for up to **1 WEEK**. Prolonged storage may degrade sampleRNA.

Dry the Slides

1. Remove slides from 100% EtOH. Leave slides for **5 MIN** at **RT**.
2. Draw 2–4 times around tissue using the Immedge™ hydrophobic barrier pen. Let the barrier dry completely **~1 MIN**.

Part 2: Tissue Pretreatment

Apply RNAscope® Hydrogen Peroxide and Protease IV

1. Add 2–4 drops/slide of RNAscope® Hydrogen Peroxide for **10 MIN** at **RT** then rinse once in distilled water.

2. Take slides from the Tissue-Tek® Slide rack, and add 2–4 drops RNAscope Protease IV to each section. Incubate for **30 MIN** at **RT.**
3. Wash slides with 1X PBS by moving the rack up and down 3-5 times and repeat with 1X PBS.

IMPORTANT! Use enough solution to completely cover the sections.

NOTE: Some tissues may require different treatment time (**15–30 MIN**) with Protease IV. Always start with **30 MIN** and adjust based on signal and morphology.

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

*RNAscope® 2.5 HD Detection Reagents-Brown User Manual, Part2 (Cat. No. 322300-USM); RNAscope® 2.5 HD Detection Reagents-Red User Manual, Part 2 (Cat. No. 322350-USM); RNAscope 2.5 HD Duplex Detection Reagents User Manual (Cat. No. 322500-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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Headquarters
7707 Gateway Blvd Suite 200, Newark, CA 94545 Phone 1-510-576-8800 Toll Free 1-877-576-3636
For support, email **support@acdbio.com**
www.acdbio.com

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