ADVANCED CELL DIAGNOSTICS

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. The required pretreat reagents are RNAscope® Hydrogen Peroxide and RNAscope Protease IV (available in RNAscope Universal Pretreatment Kit, Cat. No.322380). For Part 2 of the detection assay procedures please refer to the specific RNAscope Chromogenic Detection assay manual available on the ACD website. 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

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 for at least 3 months at **-80°C**.

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

5. The slides can be shipped in 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**.

Research Use Only. Not for diagnostic use.

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**.

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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 1 X in dH2O.
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 1XPBS 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-Plex Detection Reagents Chromogenic User Manual (Cat. No.320701_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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