ADVANCED CELL DIAGNOSTICS

Sample Preparation Technical Note for Cultured Adherent Cells Using RNAscope® 2.5 Chromogenic Assay (Single-plex and Duplex)

Introduction

This Technical Note provides guidelines for the preparation of cultured adherent cells that can be assayed using an RNAscope® Chromogenic Detection Kit (Product Cat. Nos. 322310, 322360, or 320700). The required pretreatment Reagents are RNAscope® Hydrogen Peroxide, RNAscope® Protease III (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: Cell Collection

Cell Culture

1. One day before fixation, seed cells in growth medium on chamber slides at a density that will allow cells to be 80–90% confluent at the time of fixation.

Cell Fixation

1. Remove growth media and remove the chambers.
2. Submerge the slides in a Coplin jar/staining dish containing 1X PBS.

IMPORTANT! Do not let cells dry out at any time. Always use enough solution to submerge all the cells.

3. Remove 1X PBS and add 10% Neutral Buffered Formalin (NBF). Incubate at **ROOM TEMPERATURE (RT)** for **30 MIN**.
4. Remove NBF and gently rinse slides with 1X PBS. Repeat twice.

Dehydrate and Store Cells

1. Remove final 1X PBS wash and replace with 50 mL 50% EtOH. Incubate at **RT** for **5 MIN**.
2. Remove 50% EtOH and replace with 50 mL 70% EtOH. Incubate at **RT** for **5 MIN**.
3. Remove 70% EtOH and replace with 50 mL 100% EtOH. Incubate at **RT** for **5 MIN**.
4. Remove 100% EtOH and replace with fresh 100% EtOH. Incubate at **RT** for **10 MIN**.

NOTE: The slides can be stored in 100% EtOH at **-20°C** for up to **6 MONTHS**.

Part 2: Cell Pretreatment

Rehydrate Cells

1. Submerge slides in 70% EtOH. Incubate at **RT** for **2 MIN**.

IMPORTANT! Do not let cells dry out at any time. Always use enough solution to submerge all the cells.

2. Remove 70% EtOH and replace with 50% EtOH. Incubate at **RT** for **2 MIN**.
3. Remove 50% EtOH and replace with 1X PBS. Incubate at **RT** for **10 MIN**.

Create a Hydrophobic Barrier

1. Draw 2–4 times around each well/circle on the chambered slides using the Immedge™ hydrophobic barrier pen. Let the barrier dry completely **~1 MIN**.

   NOTE: Do not let the cells dry out during this step. Place slides back into 1X PBS if the cells look too dry.

2. Rinse slides briefly with 1X PBS in a Coplin jar or staining dish.

Apply RNAscope® Hydrogen Peroxide

1. One at a time, remove each slide from the 1X PBS and tap/ and/ or flick to remove excess liquid. Place the slides on the HybEZ™ or ACD EZ-Batch™ Slide Rack and place rack in the Humidity Control Tray.
2. Add 2–4 drops of Hydrogen Peroxide to completely cover each well/circle.
3. Close the Humidity Control Tray and incubate for **10 MIN** at **RT**.
4. One at a time, take each slide from the HybEZ™ or ACD EZ-Batch™ Slide Rack and tap/flick to remove excess liquid. Submerge slides in 1X PBS.
5. Wash the slides by agitating them in the 1X PBS. Repeat with fresh 1X PBS.

Apply RNAscope® Protease III

1. One at a time, remove each slide from the 1X PBS and tap/ and/ or flick to remove excess liquid. Place the slides on the HybEZ™ Slide Rack and place rack in the Humidity Control Tray.
2. Add 2–4 drops diluted Protease III to completely cover each well/circle.

   NOTE: For most cell lines, dilute Protease III **1:15** with 1X PBS. Protease III dilution factor must be empirically determined for each new cell type

3. Close the Humidity Control Tray and incubate for **10 MIN** at **RT**.
4. One at a time, take each slide from the HybEZ™ or ACD EZ-Batch™ Slide Rack and tap/flick to remove excess liquid. Submerge slides in 1X PBS.
5. Wash the slides by agitating them in the 1X PBS. Repeat with fresh 1X PBS.

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

* RNAscope® 2.5 HD Detection Reagents -Brown User Manual, Part 2 (Doc. No. 322300-USM); RNAscope® 2.5 HD Detection Reagents-Red User Manual, Part 2 (Doc. No. 322350-USM); RNAscope 2-Plex Detection Reagents -Chromogenic User Manual (Doc. No.320494)

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.

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.

Headquarters

3960 Point Eden Way | Hayward, CA 94545 | Phone 1-510-576-8800 Toll Free 1-877-576-3636

For support, email support@acdbio.com.

www.acdbio.com