RNAscope® Fluorescent Assay for Cultured Adherent Cells (Part 1)

Introduction

This Technical Note provides guidelines for the preparation of cultured adherent cells that can be assayed using an RNAscope® Multiplex Fluorescent Assay (Cat. No. 320850). The required RNAscope® Pretreat Reagent is Pretreat 3 (available in Cat. No. 320842). For every chemical, read the Material Safety Data Sheet (MSDS) and follow handling instructions. For the latest service and support information, go to: **www.acdbio.com/support**

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 dissemble 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 Pretreat 3

1. One at a time, remove each slide from the 1X PBS and tap/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 Pretreat 3 to completely cover each well/circle.

NOTE: For most cell lines, dilute protease **1:15** with 1X PBS. Protease 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™ 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 *RNAscope® Fluorescent Multiplex Kit User Manual Part 2* (Catalog No. 320293) available at www.acdbio.com/support/technical-doc.

Obtaining Support

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

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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Unlock your RNA with RNAscope® Technology

RNAscope® allows single-cell gene expression profiling *in situ*—unlocking the full potential of RNA biomarkers. The targeted molecular signature of every cell in a sample is revealed and measured precisely—all within the intricate cellular and tissue architecture of clinical specimens.

RNAscope® is the only technology that has the sensitivity to detect every gene in the human transcriptome *in situ*, and to simultaneously quantify multiple mRNA transcripts at a single cell level. This represents a game-changing breakthrough previously unattainable by any other approach.

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