Preparing Cultured Adherent Cells for the RNAscope® Fluorescent Multiplex Assay

Introduction

This Technical Note provides guidelines to prepare cultured adherent cells for the RNAscope® Fluorescent Multiplex Assay (Cat. No. 320850). The required reagent is RNAscope® Protease III (available in RNAscope® Protease III and Protease IV Reagents, Cat. No. 322340 or RNAscope® Universal Pretreatment Kit Cat No 322380). Read the Safety Data Sheet (SDS) available on the website and follow handling instructions. Wear appropriate protective eyewear, clothing, and gloves. For the latest services 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 (four - well glass slides from Fisher Scientific, cat# 08-774-25) at a density that will allow cells to be 80–90% confluent at the time of fixation.

Cell Fixation

1. Remove growth media and disassemble chambers.
2. Submerge the slides in a Coplin jar/ Tissue-Tek® container with 200 mL of 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 Tissue-Tek® container.

Apply RNAscope® Protease III

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 Protease III to completely cover each well/circle.

   NOTE: For most cell lines, freshly 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 **http://www.acdbio.com/technical-support/user-manuals.**

Obtaining Support

For the latest services and support information, go to: **https://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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