

Cultured Adherent Cell Sample Preparation for the RNAscope™ Multiplex Fluorescent v2 Assays

Introduction

This Technical Note provides guidelines for the preparation of cultured adherent cells that can be assayed using the RNAscope™ Multiplex Fluorescent Kit v2 (Cat. No. 323100). To pretreat the cells, use RNAscope™ Protease III (available in the 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 in the document download center of each product page and follow handling instructions. Wear appropriate protective eyewear, clothing, and gloves. For the latest services and support information, contact our Technical Services.

Part 1: Collect Cells

Harvest Cells

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 disassemble chambers.
2. Submerge the slides in a Tissue-Tek® container with 200 mL of 1X PBS.
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.

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

Dehydrate and Store Cells

5. Remove 1X PBS wash, and replace with 50 mL 50% EtOH. Incubate at RT for 5 MIN.
6. Remove 50% EtOH, and replace with 50 mL 70% EtOH. Incubate at RT for 5 MIN.
7. Remove 70% EtOH, and replace with 50 mL 100% EtOH. Incubate at RT for 5 MIN.
8. 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

9. Submerge slides in 70% EtOH. Incubate at RT for 2 MIN.
10. Remove 70% EtOH, and replace with 50% EtOH. Incubate at RT for 2 MIN.
11. Remove 50% EtOH, and replace with 1X PBS. Incubate at RT for 10 MIN.

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

Apply RNAscope™ Hydrogen Peroxide

1. Lay slides on the bench, and add ~5–8 drops of RNAscope™ Hydrogen Peroxide to cover the entire section.
2. Incubate the slides at RT for 10 MIN.
3. Remove the RNAscope™ Hydrogen Peroxide solution from one slide at a time by tapping and/or flicking the slide on absorbent paper. Immediately insert the slide into a Tissue-Tek® Slide Rack submerged in a Tissue-Tek® Staining Dish filled with distilled water.
4. Wash slides 3–5 times by moving the Tissue-Tek® Slide Rack up and down in the distilled water.
5. Repeat Step 4 with fresh distilled water.

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

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

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.
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.

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

IMPORTANT: Proceed to the RNAscope™ protocol using the RNAscope™ Multiplex Fluorescent Kit User Manual Part 2 (Catalog No. 323100) available in the documents download center of each product page.

For the latest services and support information, contact our Technical Services.

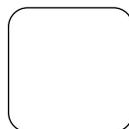
At the website, you can:

1. 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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NOTICE TO PURCHASER: PLEASE REFER TO THE RNAscope™ FLUORESCENT MULTIPLEX V2 ASSAY-USER MANUAL FOR LIMITED USE LABEL LICENSE OR DISCLAIMER INFORMATION.

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