

Sample Preparation Technical Note for Non-Adherent Cells using the RNAscope[®] 2.5 and BaseScope[™] Chromogenic Assays

Introduction

This Technical Note provides guidelines for the preparation of non-adherent cells that can be assayed using an RNAscope[®] Chromogenic Detection Kit. The required pretreat reagents are RNAscope[®] Hydrogen Peroxide and RNAscope[®] Protease III (available in RNAscope[®] Universal Pretreatment Kit No. 322380).

Part 1: Prepare Samples

Non-adherent Cell Collection

1. Harvest the cells by centrifuging at **RT** at 250 RCF for **10 MIN** in a 50 mL polypropylene tube.
2. Aspirate the supernatant without disturbing the cell pellet.
3. Wash with 40 mL 1X PBS by resuspending the cells and centrifuging at **RT** at 250 RCF.
4. Aspirate the supernatant, leaving as little liquid as possible without touching the cell pellet

Cell Fixation

1. Resuspend the cells in 5 mL of 10% NBF or 4% PFA. Gently pipette up and down 10 times to completely break apart the cell pellet.
2. Incubate the tube in a **37°C** water bath for **1 HR**.

Post-Fixation Wash and Storage

1. Centrifuge at 250 RCF for **10 MIN** to pellet the cells.
2. Remove the supernatant without disturbing the pellet.
3. Resuspend the cells in 10 mL 1X PBS, and centrifuge at 250 RCF for **10 MIN**.
4. Resuspend the cells in 10 mL 70% EtOH. Pipette up and down 10 times to completely break apart the cell pellet.

Refer to the user Safety Data Sheet (SDS) available on the ACD website. Materials required, but not provided by ACD, include 100% EtOH, 1X PBS, Superfrost[®] Plus slides (Fisher), and fixative such as 10% Neutral Buffered Formalin (10% NBF) or 4% paraformaldehyde (4% PFA).

5. Incubate at **RT** for **10 MIN** and transfer to **4°C**.

Note: The cells can be stored in 70% EtOH at **4°C** for up to **7 days**.

Slide Preparation

1. Adjust the cell density with 1X PBS to 1 x 10⁶ cells/mL.
2. Mix well by pipetting. Transfer 1 mL cell suspension to each pre-assembled cyto-centrifuge cartridge.

Note: Cell density and volume described here is based on the Hettich cyto-centrifuge with an 8 mL funnel chamber. If other cyto-centrifuge systems are used, adjust the cell density and volume to achieve a single cell layer after cyto-centrifuge.

3. Cyto-centrifuge at 800 RCF for **20 MIN**.
4. Carefully remove the supernatant completely with the pipette and disassemble the cyto-centrifuge cartridge.
5. Air dry the slides for **20 MIN**.
6. Immerse the slides in 50% EtOH. Incubate at **RT** for **5 MIN**.
7. Remove 50% EtOH and replace with 70% EtOH. Incubate at **RT** for **5 MIN**.

- Remove 70% EtOH and replace with 100% EtOH. Incubate at **RT** for **5 MIN**.
- Remove 100% EtOH and replace with fresh 100% EtOH. Incubate at **RT** for **5 MIN**.

Note: The slides can be stored in 100% EtOH at **-20°C** for up to **1 MONTH**.

Part 2: RNAscope® Pretreatment

Prepare Materials

- Bring the HybEZ™ Oven to **40°C**.
- Place a wet humidifying paper in the Humidity Control Tray, leaving the ACD EZ-Batch™ Slide Rack on the bench. Re-insert the covered tray into the oven and close the oven door. The tray should be pre-warmed for at least **20 MIN** before use.

Create a Hydrophobic Barrier

- Remove the slides from 100% EtOH and dry at **37°C** for **30 MIN** on a slide warmer.
- Draw 2–4 times around the cell spot using the Immedge™ hydrophobic barrier pen. Let the barrier dry completely **~1 MIN**.

Add RNAscope® Hydrogen Peroxide

- Add 2–4 drops Hydrogen Peroxide for **10 MIN** at **RT**. Use enough solution to completely cover the cell spot.

Note: Avoid dropping solution directly onto the cell spot to minimize cell loss. Instead, add reagent around the edge of the cell spot.

- One at a time, tap/flick each slide to remove excess liquid and submerge in 1X PBS.

- Remove 1X PBS, replace with fresh 1X PBS, and wash at **RT** for **1 MIN**.

Add RNAscope® Protease III

- One at a time, remove each slide from the 1X PBS and tap/flick to remove excess liquid. Place the slides in the ACD EZ-Batch™ Slide Rack.
- Add 2–4 drops Protease III. Use enough solution to completely cover the cell spot.
- Place the slide rack in the pre-warmed Humidity Control Tray, close lid, and incubate the tray in the HybEZ™ Oven for **30 MIN** at **40°C**.
- Take the slides out of the oven and tap/flick to remove excess Protease III. Do not let the sample dry out.
- Wash the slides in the clear EZ-Batch™ Slide Tray containing 1X PBS.
- Remove 1X PBS, replace with fresh 1X PBS, and wash the slides at **RT** for **1 MIN**.

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

* RNAscope® 2.5 HD Detection Kit-Brown User Manual (Doc. No.322310); RNAscope® 2.5 HD Detection Kit-Red User Manual (Doc. No. 322360); RNAscope Duplex Detection Kit-Chromogenic User Manual (Doc. No.322500) ; BaseScope™ Detection Kit-Brown User Manual (Doc. No. 323800) ; BaseScope™ Detection Kit-Red User Manual (Doc. No. 323900)

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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