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).

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).

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.
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 10^6 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**.

8. Remove 70% EtOH and replace with 100% EtOH. Incubate at **RT** for **5 MIN**.
9. 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

1. Bring the HybEZ™ Oven to **40°C**.
2. 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

1. Remove the slides from 100% EtOH and dry at **37°C** for **30 MIN** on a slide warmer.
2. 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

1. 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.

2. One at a time, tap/flick each slide to remove excess liquid and submerge in 1X PBS.
3. Remove 1X PBS, replace with fresh 1X PBS, and wash at **RT** for **1 MIN**.

Add 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 in the ACD EZ-Batch™ Slide Rack.
2. Add 2–4 drops Protease III. Use enough solution to completely cover the cell spot.
3. 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**.
4. Take the slides out of the oven and tap/flick to remove excess Protease III. Do not let the sample dry out.
5. Wash the slides in the clear EZ-Batch™ Slide Tray containing 1X PBS.
6. 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

Headquarters
7707 Gateway Blvd Suite 200, Newark, CA 94545 Phone 1-510-576-8800 Toll Free 1-877-576-3636
For support, email **support.acd@bio-techne.com**
www.acdbio.com

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