

Sample Preparation Technical Note for Non-Adherent Cells using the RNAscope® Fluorescent Multiplex Assay

Introduction

This Technical Note provides guidelines to prepare non-adherent cells that can be assayed using the RNAscope® Fluorescent Multiplex Detection Kit. The required RNAscope® Pretreatment Reagent is Protease III (available in RNAscope® Protease III and Protease IV Reagents, Cat No. 322340). 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 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 1×10^6 cells/mL.
2. Mix well by pipetting. Transfer 1 mL cell suspension to each pre-assembled cyto-centrifuge cartridge.

Note: The cell density and volume described are 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 Protease III

1. Place the slides in the 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 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 *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

For Research Use Only .Not for diagnostic use.

NOTICE TO PURCHASER: PLEASE REFER TO THE RNASCOPE® FLUORESCENT MULTIPLEX USER MANUAL FOR LIMITED USE LABEL LICENSE OR DISCLAIMER INFORMATION. Advanced Cell Diagnostics, Inc. reserves the right to change its products and services at any time to incorporate technological developments. This manual is subject to change without notice. Although this manual has been prepared with every precaution to ensure accuracy, Advanced Cell Diagnostics, Inc. assumes no liability for any errors, omissions, or for any damages resulting from the use of this information.

© 2018 Advanced Cell Diagnostics. All rights reserved. RNAscope® and HybEZ™ are trademarks of Advanced Cell Diagnostics. Diagnostics, Inc. All other trademarks belong to their respective owners.

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

