# Preparing FFPE Cell Pellets for the RNAscope® and BaseScope™ Assays

## Introduction

This Technical Note provides guidelines to prepare formalin-fixed, paraffin-embedded (FFPE) cell pellets for the RNAscope® 2.5 and BaseScope™ Assays.

Wear appropriate protective eyewear, clothing, and gloves. For the latest services and support information, go to: **www.acdbio.com/support.**

## Workflow

### Part 1: Collect Cells

#### *Harvest Cells*

1. Spin down 10–20 million cells in a 50 mL conical tube (Recommended speed: 250g for **10 MIN**).
2. Suspend cells with 1X PBS and spin down again.
3. Aspirate off the 1X PBS.

#### *Fix Cells*

1. Prepare 10 mL of 10% formaldehyde in 1X PBS by adding 1 mL 10X PBS and 2.7 mL of 37% formaldehyde to 6.3 mL of distilled water.
2. Add 10 mL of fresh 10% Formaldehyde to the cell pellet.
3. Pipette up and down to suspend the cell pellet completely. Make sure that there are no cell clumps.
4. Add an additional 40 mL of 10% Formaldehyde to the conical tube.
5. Cap the tube and use a rotator to continually mix the cells at **ROOM TEMPERATURE (RT)** for **24 HR**.

#### *Wash Cells*

1. Spin the cells down and decant the 10% Formaldehyde into an appropriate waste container. Do not disturb the pellet.
2. Resuspend the cells in 10 mL of 1X PBS, and then transfer the cell suspension to a 15 mL conical tube.
3. Centrifuge the 15 mL conical tube, and aspirate off the 1X PBS. Do not disturb the pellet.

### Part 2: Embed Cells

#### *Create Pellet*

1. Warm the Histogel by placing it in a beaker with water and microwaving until the gel is liquefied, approximately **1 MIN**. Do not boil.

> **NOTE:** For the next steps (2–5), make sure that the water, tube, and pipette tip are hot. This will prevent the Histogel from solidifying during the procedure. You can also cut the pipette tip off to help in pipetting of Histogel.

2. Gently resuspend the cell pellet in 200 µL of liquid Histogel.
3. Place the 15 mL tube into an uncapped 50 mL conical tube containing hot water.
4. Centrifuge the 50 mL tube for **5 MIN**, and pipette off the clear Histogel above the cell pellet.
5. Use a 200 µL pipette to remove the Histogel cell pellet from the bottom of the 15 mL tube.
6. Place the cell pellet onto a piece of parafilm on ice to allow the pellet to solidify for **2–3 MIN**.
7. Submerge the Histogel cell pellet in 1X PBS.

**NOTE:** Do not leave the pellet in 1X PBS overnight. If needed, replace the 1X PBS with 70% EtOH. Seal the tube tightly to prevent evaporation.

### *Embed Pellet*

1. Dehydrate and embed the solid cell pellet into a paraffin block using a standard embedding procedure. We recommend the protocol as shown below. It includes three 100% EtOH steps to completely remove all water from the tissue to allow RNA preservation. The required time for each step may vary depending on the cell pellet size. Shorter or longer processing time may impact long term RNA stability.

| Step | Reagent | Time (min) | Temperature |
|---|---|---|---|
| 1 | 70% EtOH | 30–60 | 15–37°C |
| 2 | 80% EtOH | 30–60 | 15–37°C |
| 3 | 90% EtOH | 30–60 | 15–37°C |
| 4 | 95% EtOH | 30–60 | 15–37°C |
| 5 | 100% EtOH | 30–60 | 15–37°C |
| 6 | 100% EtOH | 30–60 | 15–37°C |
| 7 | 100% EtOH | 30–60 | 15–37°C |
| 8 | Xylene | 25–45 | 15–37°C |
| 9 | Xylene | 25–45 | 15–37°C |
| 10 | Xylene | 25–45 | 15–37°C |
| 11 | Paraffin | 30–60 | 57–60°C |
| 12 | Paraffin | 30–60 | 57–60°C |
| 13 | Paraffin | 30–60 | 57–60°C |

2. Process into a paraffin block and section to a thickness of 5±1 µm. Mount sections onto SuperFrost Plus slides (Fisher Scientific, cat # 12-550-15). Leave a gap of at least 3mm along the long edges and at least 5mm from the top/bottom edge. Please follow the appropriate sample pretreatment protocol depending the assay used

**IMPORTANT!** Proceed to the RNAscope® or BaseScope™ protocol using an appropriate user manual 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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