

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. Add 10 mL of fresh 10% Formaldehyde to the cell pellet.
2. Pipette up and down to suspend the cell pellet completely. Make sure that there are no cell clumps.
3. Add an additional 40 mL of 10% Formaldehyde to the conical tube.
4. 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 NBF 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.

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.
2. Prepare the sample for the RNAscope® or BaseScope™ Assay by following the procedures in the *Formalin-Fixed Paraffin-Embedded (FFPE) Sample Preparation and Pretreatment User Manual* (Document No. 322452-USM).

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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