



# RNAscope<sup>®</sup> Fluorescent Assay Sample Preparation for FFPE Tissue (Part 1)

## Introduction

This Technical Note provides guidelines for preparing and pretreating formalin-fixed, paraffin-embedded (FFPE) tissue for fluorescent detection using the RNAscope<sup>®</sup> Fluorescent Multiplex Reagent Kit (Cat. No. 320850). The required RNAscope<sup>®</sup> Pretreat Reagents are Pretreat 2 (Cat.

No. 320043) and Pretreat 4 (available in Cat. No. 320842). For every chemical, read the Material Safety Data Sheet (MSDS) and follow handling instructions. For the latest services and support information, go to: [www.acdbio.com/support](http://www.acdbio.com/support)

## Workflow

### Part 1: Prepare Slides

#### Prepare Tissue Sections

1. Fix tissue in fresh 10% NBF for **16–32 HRS** at **ROOM TEMPERATURE (RT)**.
2. Dehydrate, embed in paraffin, and cut sample into 5 +/- 1  $\mu\text{m}$  sections. Mount sections on Superfrost<sup>®</sup> Plus slides.

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**OPTIONAL STOPPING POINT 1:** Use sectioned tissue within **3 months**. Store sections with dessicants at **RT**.

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#### Bake Slides

1. Bake slides in a dry oven for **1 HR** at **60°C**.

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**OPTIONAL STOPPING POINT 2:** Use sectioned tissue within **1 week**. Store sections with dessicants at **RT**.

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#### Deparaffinize FFPE Sections

1. In a fume hood:
  - Fill two Tissue-Tek<sup>®</sup> Clearing Agent dishes with ~200 mL fresh xylene.
  - Fill two Tissue-Tek<sup>®</sup> Staining dishes with ~200 mL fresh 100% EtOH.
2. Place slides in a Tissue-Tek<sup>®</sup> Slide Rack in xylene **2 x 5 MIN**.
3. Incubate slides in 100% EtOH **2 x 1 MIN**.

4. Remove slides from rack. Air dry slides for **5 MIN** at **RT**.

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**OPTIONAL STOPPING POINT 3:** Air dry overnight at **RT** (must use within **24 HRS**) or proceed directly to the next step.

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### Part 2: Tissue Pretreatment

#### Prepare Materials

1. Bring HybEZ<sup>™</sup> Oven to **40°C**.
2. Place a wet humidifying paper in the Humidity Control Tray, leaving the HybEZ<sup>™</sup> Slide Rack on bench. Re-insert the covered tray into the oven and close the oven door. The tray should be pre-warmed for at least **30 MIN** before use. Keep tray warm during the assay.
3. Prepare 700 mL fresh 1X Pretreat 2 in a beaker. Cover with foil, bring to a mild boil, and maintain uniform boiling at **99–100°C**. Do not boil more than **30 MIN** before use.

#### Apply Pretreat 2

1. With a pair of forceps *very slowly* submerge a slide rack containing the slides into boiling 1X Pretreat 2 solution for **15 MIN**.

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**Note:** Depending on tissue type, boiling time may need to be adjusted.

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## TECHNICAL NOTE: Sample Prep

2. *Immediately* transfer hot slide rack to a staining dish containing distilled water.
3. Wash slides in the distilled water by moving the rack up and down 3–5 times. Repeat with fresh distilled water.
4. Wash slides in fresh 100% EtOH by moving the rack up and down 3–5 times. Air dry.
4. Wash the slides by moving the rack up and down 3–5 times and repeat with fresh distilled water.

### Create Barrier

1. Draw 2–4 times around tissue using the Immedge™ hydrophobic barrier pen. Let the barrier dry completely ~1 MIN or **OVERNIGHT** at RT.

## OPTIONAL STOPPING POINT 4

### Apply Pretreat 4

1. Place slides in the HybEZ™ Slide Rack, and add 2–4 drops of Pretreat 4 to each section. Use enough solution to completely cover the sections.
2. Place the HybEZ™ Slide Rack in the pre-warmed HybEZ™ Humidity Control Tray. Seal tray and insert back into the HybEZ™ Oven. Incubate at **40°C** for **30 MIN**.

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**NOTE:** If needed, prepare RNAscope® Multiplex Fluorescent assay materials during this step.

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3. Place slides in a Tissue-Tek® Slide Rack submerged in distilled water.

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**IMPORTANT!** Proceed to the RNAscope® protocol using the *RNAscope® Fluorescent Multiplex Kit User Manual Part 2* (Catalog No. 320293) available at [www.acdbio.com/support/technical-doc](http://www.acdbio.com/support/technical-doc).

**NOTE:** Specific imaging options may be required for FFPE tissue due to autofluorescence. Please refer to the user manual for guidance.

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### Obtaining Support

For the latest services and support information, go to: [www.acdbio.com/support](http://www.acdbio.com/support).

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.

## Unlock your RNA with RNAscope® Technology

RNAscope® allows single-cell gene expression profiling *in situ*—unlocking the full potential of RNA biomarkers. The targeted molecular signature of every cell in a sample is revealed and measured precisely, all within the intricate cellular and tissue architecture of clinical specimens.

**For Molecular Biology Applications (MBA), not intended for diagnosis. Refer to appropriate regulations.**

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