

# Sample Preparation Technical Note for FFPE Tissue Using RNAscope® Multiplex Fluorescent Assay

## Introduction

This Technical Note provides guidelines for preparing and pretreating formalin-fixed, paraffin-embedded (FFPE) tissue for fluorescent detection using the RNAscope® Fluorescent Multiplex Kit (Cat. No. 320850). The required RNAscope® pretreatment reagents are RNAscope® Target Retrieval and Protease III (available in RNAscope Universal

Pretreatment Kit, Cat. No 322380). Note: We recommend RNAscope® Protease III on human tissue, when using Mouse tissue or cell pellets, users can also try Protease IV, if Protease III does not yield optimal results. Refer to the Safety Data Sheet (SDS), available on the ACD website (<http://www.acdbio.com/technical-support/user-manuals>).

## 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 µm sections. Mount sections on Superfrost® Plus slides.

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**OPTIONAL STOPPING POINT 1:** Use sectioned tissue within **3 months**. Store sections with desiccants 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® Clearing Agent dishes with ~200 mL fresh xylene.
  - Fill two Tissue-Tek® Staining dishes with ~200 mL fresh 100% EtOH.
2. Place slides in a Tissue-Tek® Slide Rack in xylene **2 x 5 MIN**.
3. Incubate slides in 100% EtOH **2 x 1 MIN**.

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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™ Oven to **40°C**.
2. Place a wet humidifying paper in the Humidity Control Tray, leaving the HybEZ™ 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 Target Retrieval 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 RNAscope® Target Retrieval**

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

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

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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.

### **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**.

### **Apply RNAscope® Protease III**

1. Place slides in the HybEZ™ or ACD EZ-Batch™ Slide Rack, and add 2–4 drops of Protease III to each section. Use enough solution to completely cover the sections.
2. Place the HybEZ™ or ACD EZ-Batch™ 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.
4. Wash the slides by moving the rack up and down 3–5 times and repeat with fresh distilled water.

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**IMPORTANT!** Proceed immediately to the RNAscope® protocol using the *RNAscope® Fluorescent Multiplex Kit User Manual Part 2* (Doc no 320293) available at <http://www.acdbio.com/technical-support/user-manuals>

**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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**Note:** We recommend RNAscope® Protease III on human tissue, when using Mouse tissue or cell pellets, users can also try Protease IV, if Protease III does not yield optimal results.

### **Obtaining Support**

For the latest services and support information, go to:

<http://www.acdbio.com/technical-support/user-manuals>.

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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### **Headquarters**

3960 Point Eden Way Hayward, CA 94545 Phone 1-510-576-8800 Toll Free 1-877-576-3636

For support, email [support@acdbio.com](mailto:support@acdbio.com).

[www.acdbio.com](http://www.acdbio.com)