RNAscope® 2.0 HD Detection Kit (RED) Quick Guide

For FFPE Tissues

Catalog Number 320485

Introduction

This quick guide is intended for advanced users who are familiar with the procedures in the *Sample Preparation Pretreatment Guide for Formalin-Fixed Paraffin-Embedded (FFPE),* see Catalog No. 320511 and *RNAscope® 2.0 HD Detection Kit (RED) User Manual Part 2* (Catalog No. 320487*).* Refer to the user manual for safety guidelines. For every chemical, read the Material Safety Data Sheet (MSDS) and follow handling instructions. Wear appropriate protective eyewear, clothing, and gloves. For the latest services and support information, go to: **www.acdbio.com/support.**

Part 1 Prepare and Pretreat Samples

	Workflow Steps	
	PREPARE FFPE SECTIONS	1. Immediately place dissected tissue sample 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. OPTIONAL STOPPING POINT (1). Use sectioned tissue within 3 months. Store sections with dessicants at RT.
	PREPARE SLIDES ~1.5 HOURS Bake Slides ↓ Deparaffinize FFPE Sections	Bake Slides 1. Bake slides in a dry oven for **1 HR** at **60°C**. OPTIONAL STOPPING POINT (2). Use sectioned tissue within 1 week. Store sections with dessicants at RT. 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% ethanol. 2. Place slides in a Tissue-Tek® Slide Rack in xylene **2 x 5 MIN**. 3. Incubate slides in 100% ethanol **2 x 1 MIN**.Remove slides from rack. Air dry slides for **5 MIN** at **RT.** OPTIONAL STOPPING POINT (3). Air dry overnight at RT (must use within 24 hrs) or proceed directly to the next step.
	PRETREAT SAMPLES ~1–2 HOURS Prepare Oven and Reagents ↓ Apply Pretreat 1 ↓ Apply Pretreat 2 ↓ Create Barrier ↓	Prepare Oven and Reagents **(30 MIN at 40°C)** 1. Set HybEZ™ oven to **40°C** and warm HybEZ™ Humidity Control Tray containing wet Humidifying Paper for **30 MIN** before use. Keep tray warm during assay. 2. Prepare 700 mL fresh 1X Pretreat 2 in a beaker. Cover with foil, bring to a mild boil, and maintain. Do not boil more than **30 MIN** before use. Apply Pretreat 1 **(10 MIN at RT)** 1. Add ~5–8 drops of Pretreat 1 to each section for **10 MIN** at **RT**. 2. Place slides into aTissue-Tek® Slide Rack submerged in distilled water. 3. Wash slides in the distilled water by moving the rack up and down 3–5 times and repeat with fresh distilled water.

	Apply Pretreat 3	Apply Pretreat 2 1. With a pair of forceps *very slowly* submerge the slide rack into boiling 1X Pretreat 2 solution. Refer to **Appendix A** of the *Part 1, Sample Preparation and Pretreatment Guide* (Cat. No. 320511) for FFPE Tissue for specific pretreatment time, depending on your tissue type. 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 and repeat with fresh distilled water. 4. Wash slides in fresh **100% EtOH** by moving the rack up and down 3–5 times, and air dry. Create Barrier 1. Draw 2–4 times around tissue using the Immedge™ hydrophobic barrier pen. Dry completely ~**2 MIN** or **OVERNIGHT** at **RT**. Apply Pretreat 3 1. Remove excess liquid from slides, place in the HybEZ™ Slide Rack, and add ~5 Drops of Pretreat 3 to each section. 2. Place the HybEZ™ Slide Rack in the prewarmed HybEZ™ Humidity Control Tray. Seal tray and insert back into the HybEZ™ Oven. Incubate at **40°C** for **30 MIN**. **Note:** If needed, prepare RNAscope® 2.0 assay materials during this step. 3. Wash slides in the distilled water by moving the rack up and down 3–5 times and repeat with fresh distilled water.

Part 2: RNAscope® 2.0 Assay

	Workflow Steps	
	PREPARE THE MATERIALS ~10–30 MINUTES	1. Prepare **3 L** of **1X WASH BUFFER** by adding 2.94 L distilled water and 1 bottle (60 mL) of 50X Wash Buffer to a large carboy. Mix well. 2. Prepare **50% HEMATOXYLIN** and **0.02% AMMONIA WATER**. 3. Add **200 mL XYLENE** to a Tissue Tek® Clearing Agent Dish. 4. Equilibrate reagents and equipment: • Place **AMP 1–6** at **RT**. • Warm probes for **10 MIN** at **40°C** and cool to **RT**.
	RUN THE ASSAY ~5 HOURS Hybridize Probe ↓ Hybridize Amp 1 ↓ Hybridize Amp 2 ↓ Hybridize Amp 3 ↓ Hybridize Amp 4 ↓ Hybridize Amp 5 ↓ Hybridize Amp 6 ↓ Detect the Signal ↓	Hybridize Probe (**2 HRS at 40°C**) 1. Remove excess liquid from slides, place in the HybEZ™ Slide Rack, and add ~4 drops probe to each section. 2. Insert sealed tray containing HybEZ™ Slide Rack back into the HybEZ™ Oven for **2 HRS** at **40°C**. Remove slide rack. 3. Wash slides in 1X Wash Buffer for **2 MIN** at **RT**. Repeat with fresh 1X Wash Buffer. Hybridize Amp 1 (**30 MIN at 40°C**) 1. Remove excess liquid from slides, place in the HybEZ™ Slide Rack, and add ~4 drops **AMP 1** to each section. 2. Insert sealed tray containing HybEZ™ Slide Rack into the HybEZ™ Oven for **30 MIN** at **40°C**. Remove slide rack. 3. Wash slides in 1X Wash Buffer for **2 MIN** at **RT**. Repeat with fresh buffer. Hybridize Amp 2 (**15 MIN at 40°C**) 1. Remove excess liquid from slides, place in the HybEZ™ Slide Rack, and add ~4 drops **AMP 2** to each section.

	Counterstain the Slides ↓ Mount the Slides	2. Insert sealed tray containing HybEZ™ Slide Rack into the HybEZ™ Oven for **15 MIN** at **40°C**. Remove slide rack. 3. Wash slides in 1X Wash Buffer for **2 MIN** at **RT**. Repeat with fresh buffer. Hybridize Amp 3 (**30 MIN at 40°C**) 1. Remove excess liquid from slides, place in the HybEZ™ Slide Rack, and add ~4 drops **AMP 3** to each section. 2. Insert sealed tray containing HybEZ™ Slide Rack into the HybEZ™ Oven for **30 MIN** at **40°C**. Remove slide rack. 3. Wash slides in 1X Wash Buffer for **2 MIN** at **RT**. Repeat with fresh buffer. Hybridize Amp 4 (**15 MIN at 40°C**) 1. Remove excess liquid from slides, place in the HybEZ™ Slide Rack, and add ~4 drops **AMP 4** to each section. 2. Insert sealed tray containing HybEZ™ Slide Rack into the HybEZ™ Oven for **15 MIN** at **40°C**. Remove slide rack, but do *not* place tray back into the oven. 3. Wash slides in 1X Wash Buffer for **2 MIN** at **RT**. Repeat with fresh buffer. Hybridize Amp 5 (**30 MIN at RT**) 1. Remove excess liquid from slides, place in the HybEZ™ Slide Rack, and add ~4 drops **AMP 5** to each section. 2. Incubate sealed tray containing HybEZ™ Slide Rack for **30 MIN** at **RT**. 3. Wash slides in 1X Wash Buffer for **2 MIN** at **RT**. Repeat with fresh buffer. Hybridize Amp 6 (**15 MIN at RT**) 1. Remove excess liquid from slides, place in the HybEZ™ Slide Rack, and add ~4 drops **AMP 6** to each section. 2. Incubate sealed tray containing HybEZ™ Slide Rack for **15 MIN** at **RT**. 3. Wash slides in 1X Wash Buffer for **2 MIN** at **RT**. Repeat with fresh buffer. Detect the Signal (**10 MIN at RT**) 1. Briefly spin RED-B and mix 1 volume of RED-B to 60 volumes of RED-A. 2. Remove excess liquid from slides, place in the HybEZ™ Slide Rack, and pipette ~120 µL of RED solution onto each tissue section. 3. Incubate sealed tray containing HybEZ™ Slide Rack for **10 MIN** at **RT**. 4. Remove solution from slides and wash 3–5 times in distilled water. Counterstain the Slides (**2 MIN at RT**) 1. Place slides in 50% Hematoxylin I for **2 MIN** at **RT.** Wash 3–5 times in distilled water and repeat with fresh distilled water. 2. Wash slides **10 SEC** in 0.02% ammonia water, and then wash 3–5 times in distilled water. Mount the Slides 1. Dry slides in a **60°C** dry oven for **15 MIN**. 2. Cool the slides at **RT** ~**5 MIN**. 3. Dip the slides into fresh pure xylene and immediately place 1–2 drops of EcoMount on the slide before the xylene dries. Place coverslip over section**.** 4. Air dry for **5 MIN**.
	EVALUATE THE RESULTS	Examine tissue sections under a standard bright field microscope at 20–40X magnification.

Troubleshooting

For troubleshooting information, please contact technical support at **support@acdbio.com**.

For Molecular Biology Applications, not intended for diagnosis.

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