miRNAscope™ HD Reagent Kit (RED)

Introduction

This quick guide is intended for advanced users who are familiar with the procedures in the *miRNAscope™ HD (RED) Assay User Manual* (Document No. UM 324510). Refer to the user manual for safety guidelines. For every chemical, read the Safety Data Sheet (SDS) and follow handling instructions. Wear appropriate protective eyewear, clothing, and gloves. For the latest services and support information, go to: **https://acdbio.com/technical-support/support-overview.**

Part 1: Prepare and Pretreat Samples

	Workflow Steps	
	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 (1). Use sectioned tissue within 1 week. Store sections with desiccants at room temperature (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% alcohol. 2. Place slides in a Tissue-Tek® Slide Rack in xylene **2 x 5 MIN**. 3. Incubate slides in 100% alcohol **2 x 2 MIN**. 4. Remove slides from rack. Dry slides for **5 MIN** at **60°C** or until completely dry.
	POST-FIX SAMPLES 2 HOURS or OVERNIGHT	1. In a fume hood, do one of the following: • Incubate slides in fresh10% NBF **OVERNIGHT (~16 HRS)** at **RT** or • Incubate slides in fresh 12% formaldehyde for **2 HRS** at **RT** 2. Remove slides from fix and wash them for **2 MIN** in distilled water. 3. Dry slides for **5 MIN** at **60°C** or until completely dry.
	PRETREAT SAMPLES ~1–2 HOURS Prepare Oven and Reagents ↓ Apply Hydrogen Peroxide ↓ Apply Target Retrieval ↓ Create Barrier ↓ Apply Protease III	Prepare Oven and Reagents **(30 MIN at 40°C)** 4. Set HybEZ™ oven to **40°C** and warm HybEZ™ Humidity Control Tray containing wet humidifying paper for **30 MIN** before use. 5. Prepare 200 mL fresh 1X Target Retrieval Reagent by adding 180 mL distilled water to 20 mL of 10X Target Retrieval Reagents. Mix well. 6. Turn on the steamer, and set the heating time to **95 MIN**. Allow the temperature to rise to at least **99°C**. Apply Hydrogen Peroxide **(10 MIN at RT)** 1. Add ~5–8 drops of hydrogen peroxide to each section for **10 MIN** at **RT**. 2. Remove the hydrogen peroxide, and place the slides in a slide rack. 3. Wash the slides by moving the rack up and down 3–5 times in a staining dish filled with distilled water. Repeat with fresh water. Apply Target Retrieval **(15–30 MIN at boiling point)** 1. With a pair of forceps slowly submerge the slide rack into boiling 1X Target retrieval solution for **15 MIN**. Refer to **Appendix A** of the user manual (Doc. No. UM 324510) to determine the appropriate conditions for your tissue type. 2. Wash the slides by moving the rack up and down 3–5 times in a staining dish filled with distilled water. Repeat with fresh water.

		3. Wash slides in100% alcohol, then air dry. Create Barrier 1. Once the slides are dry, draw 2–4 times around the tissue using the Immedge™ hydrophobic barrier pen. Dry completely ≥ **5 MIN** at **RT**. OPTIONAL STOPPING POINT (2). Dry slides overnight for use the following day or proceed directly to the next section. Apply Protease III **(15–30 MIN at 40°C)** 1. Place the slides in the ACD EZ-Batch™ Slide Holder, and then add ~4 drops of protease to cover each section. 2. Place the slide holder into the pre-warmed humidity control tray. Seal the tray. • For HeLa cell pellets, incubate slides for **15 MIN** at **40°C** in the HybEZ™ oven. • For cell pellets, incubate slides for **15 MIN** at **RT**. • For tissues, incubate slides for **30 MIN** at **40°C** in the HybEZ™ oven. 3. Place the slide holder with the slides into the ACD EZ-Batch™ Wash Tray filled with fresh distilled water. Wash the slides with slight agitation.

Part 2: Run the Assay

	Workflow Steps	
	PREPARE THE MATERIALS ~10–30 MIN	1. Warm 50X Wash Buffer for **10–20 MIN** before preparing 1X Wash Buffer solution. 2. Prepare 5 L of 1X Wash Buffer by adding 4.90 L distilled water and 100 mL of 50X Wash Buffer to a large carboy. Mix well. 3. Prepare 50% Gill's Hematoxylin-I and 0.02% Ammonium Hydroxide. 4. Equilibrate reagents: • Place Amps 1–6 at **RT**. • Equilibrate probes for **15 MIN** at **40°C**.
	RUN THE ASSAY ~8 HOURS Hybridize Probe ↓ Hybridize Amp 1 ↓ Hybridize Amp 2 ↓ Hybridize Amp 3 ↓ Hybridize Amp 4 ↓ Hybridize Amp 5 ↓ Hybridize Amp 6 ↓ Detect the Red Signal ↓ Counterstain the Slides ↓ Mount the Slides	Hybridize Probe **(2 HRS at 40°C)** 1. Remove excess liquid from slides, place in the ACD EZ-Batch™ Slide Holder, and add ~4 drops of probe to each section. 2. Insert sealed tray containing the slide holder into the HybEZ™ Oven for **2 HRS** at **40°C**. 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 ACD EZ-Batch™ Slide Holder, and add ~4 drops Amp 1 to each section. 2. Insert sealed tray containing the slide holder 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 1X Wash Buffer. Hybridize Amp 2 **(15 MIN at 40°C)** 1. Remove excess liquid from slides, place in the ACD EZ-Batch™ Slide Holder, and add ~4 drops Amp 2 to each section. 2. Insert sealed tray containing the slide holder 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 ACD EZ-Batch™ Slide Holder, and add ~4 drops Amp 3 to each section.

	Workflow Steps	
	↓ Evaluate the Results	2. Insert sealed tray containing the slide holder 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 ACD EZ-Batch™ Slide Holder, and add ~4 drops Amp 4 to each section. 2. Insert sealed tray containing the slide holder into the HybEZ™ Oven for **15 MIN** at **40°C**. 3. Wash slides in 1X Wash Buffer for **2 MIN** at **RT**. Repeat with fresh buffer. **IMPORTANT!** Do not insert tray into HybEZ™ Oven for the rest of the procedure. Hybridize Amp 5 **(30 MIN at RT)** 1. Remove excess liquid from slides, place in the ACD EZ-Batch™ Slide Holder, and add ~4 drops Amp 5 to each section. 2. Incubate the sealed tray containing the slide holder 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 ACD EZ-Batch™ Slide Holder, and add ~4 drops Amp 6 to each section. 2. Incubate the sealed tray containing the slide holder for for **15 MIN** at **RT**. 3. Wash slides in 1X Wash Buffer for **2 MIN** at **RT**. Repeat with fresh buffer. Detect the Red Signal **(10 MIN at RT)** 1. Briefly spin Red-B and mix 1 volume of Red -B to 60 volumes of Red -A (use within **3–5 MIN**). 2. Remove excess liquid from slides, place in the ACD EZ-Batch™ Slide Holder, and pipette ~120 µL of Red solution onto each tissue section. 3. Incubate sealed tray containing the slide holder for **10 MIN** at **RT**. 4. Place slides in a Tissue-Tek® Slide Rack, and wash the slides by moving the rack up and down 3–5 times in a staining dish filled with distilled water. Counterstain the Slides 1. Place slides in 50% Hematoxylin for **2 MIN** at **RT.** 2. Wash the slides by moving the rack up and down 3–5 times in distilled water. Repeat with fresh distilled water. 3. Place slides in 0.02% Ammonium Hydroxide for **10 SEC.** 4. Wash the slides by moving the rack up and down 3–5 times in distilled water. Mount the Slides 1. Dry slides in a **60°C** dry oven for **15–30 MIN**. 2. Dip the slides into fresh pure xylene until the hydrophobic barrier disappears and immediately place 1–2 drops of EcoMount on the slides before the xylene dries. Place a coverslip over the section. 3. Before viewing under the microscope, dry slides for at least **30 MIN** or until the xylene completely evaporates. Evaluate the Results 1. Examine tissue sections under a standard bright field microscope at 20–40X magnification.

Troubleshooting

For troubleshooting information, please contact technical support at **support.acd@bio-techne.com**.

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