QUICK GUIDE: RNAscope® 2.5 BROWN – **PART 2**

**Tissue Pretreatment (Part 2)**

***Prepare Sections***

1. **Turn on HybEZTM oven** and switch toor ensure **oven** is in **“Hold T” mode** and set at **60°C**.
2. Bake slides for **60 MIN** at **60°C** in oven tray without humidifying paper.

***Prepare Materials***

1. Reduce HybEZTM Oven temperature to **40°C** by switching to **“RNAscope” mode**. Place a wet humidifying paper in the Humidity Control Tray and re-insert covered tray into oven.

***Apply RNAscope® Protease III***

1. Once oven reaches 40°C, place slides in HybEZTM Slide Rack, and **add 2-4 drops** of **Protease III** to each section. Use enough solution to completely cover the sections.
2. Place the HybEZTM Slide Rack in the prewarmed oven. Incubate at **40°C** for **30 MIN**.

\*\* *Prepare RNAscope****®*** *2.5 assay materials during this step.*

1. Wash slides in clear EZ-Batch Wash Tray by **submerging** them in **dH2O** and tilting tray back and forth 3-5 times. **Repeat** with **fresh dH2O**.

RNAscope® 2.5 Assay

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| Workflow Steps | |
| PREPARE THE MATERIALS  ~10-30 MIN | 1. Prepare 1.1L of 1X Wash Buffer by adding 1078 mL dH2O and 22 mL of 50X Wash Buffer to a 1L glass bottle. Mix well. 2. Equilibrate reagents and equipment:  * Remove Amp 1-6 from refrigerator. * Warm probes by placing bottles atop the preheated HybEZTM Oven, then cool to **RT** prior to use. |
| 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  ↓  Counterstain the Slides  ↓  Mount the Slides | Hybridize Probe (**2 HRS** at **40°C**)   1. Remove excess liquid from slides and **add ~40µL** of **probe** to each section. 2. Insert sealed tray containing HybEZTM Slide Rack back into HybEZTM Oven for **2 HRS** at **40°C**. Remove slide rack. 3. Wash slides in 1X Wash Buffer by tilting the tray 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 and **add 2-4 drops** of **Amp 1** to each section. 2. Insert sealed tray containing HybEZTM Slide Rack back into HybEZTM Oven for **30 MIN** at **40°C**. Remove slide rack. 3. Wash slides in 1X Wash Buffer by tilting the tray 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 and **add 2-4 drops** of **Amp 2** to each section. 2. Insert sealed tray containing HybEZTM Slide Rack back into HybEZTM Oven for **15 MIN** at **40°C**. Remove slide rack. 3. Wash slides in 1X Wash Buffer by tilting the tray for **2 MIN** at **RT**. Repeat with fresh 1X Wash Buffer. |
| Hybridize Amp 3 (**30** **MIN** at **40°C**)   1. Remove excess liquid from slides and **add 2-4 drops** of **Amp 3** to each section. 2. Insert sealed tray containing HybEZTM Slide Rack back into HybEZTM Oven for **30 MIN** at **40°C**. Remove slide rack. 3. Wash slides in 1X Wash Buffer by tilting the tray for **2 MIN** at **RT**. Repeat with fresh 1X Wash Buffer. |
| Hybridize Amp 4 (**15** **MIN** at **40°C**)   1. Remove excess liquid from slides and **add 2-4 drops** of **Amp 4** to each section. 2. Insert sealed tray containing HybEZTM Slide Rack back into HybEZTM Oven for **15 MIN** at **40°C**. Remove slide rack but do ***not*** place tray back into oven. 3. Wash slides in 1X Wash Buffer by tilting the tray 3-5 times every 30 seconds for **2 MIN** at **RT**. Repeat with fresh 1X Wash Buffer. |
| Hybridize Amp 5 (**30** **MIN** at **RT**)   1. Remove excess liquid from slides and **add 2-4 drops** of **Amp 5** to each section. 2. Incubate sealed tray containing HybEZTM Slide Rack for **30 MIN** at **RT**. 3. Wash slides in 1X Wash Buffer by tilting the tray 3-5 times every 30 seconds for **2 MIN** at **RT**. Repeat with fresh 1X Wash Buffer. |
| Hybridize Amp 6 (**15** **MIN** at **RT**)   1. Remove excess liquid from slides and **add 2-4 drops** of **Amp 6** to each section. 2. Incubate sealed tray containing HybEZTM Slide Rack for **15 MIN** at **RT**. 3. Wash slides in 1X Wash Buffer by tilting the tray 3-5 times every 30 seconds for **2 MIN** at **RT**. Repeat with fresh 1X Wash Buffer. |
| Detect the Signal (**10** **MIN** at **RT**)   1. Mix equal volumes of BROWN-A and BROWN-B (pipette 20 µL/section for each one). 2. Remove excess liquid from slides and **pipette 40 µL** of **DAB** onto each tissue section. 3. Incubate sealed tray containing HybEZTM Slide Rack for **10 MIN** at **RT**. 4. Remove DAB from slides and wash 3-5 times in **dH2O**. |
| Counterstain the Slides   1. Follow Nissl protocol for RNAscope outlined in wet lab recipe/protocol binder (also on lab webpage).   Mount the Slides   1. Use 3-4 drops of Permount and coverslip, let air dry overnight at RT. |