

# RNAscope® FFPE Assay Quick Guide

**Prior to Assay:** Prepare 1X **Pretreat 2** (& heat to boiling in a beaker) & 1X **Wash Buffer**. Pre-warm **Target Probe, Amp 1, Amp 2, Amp 3, and Amp 4** to 40°C and leave them @ RT. Bring HybEZ™ Oven and Humidity Control Tray to 40°C.

<b>1</b>	<b>De-Paraffinization</b>	<ol style="list-style-type: none"> <li>1) <b>Xylene</b> 2 X 5 min</li> <li>2) 100% <b>ethanol</b> 2 X 3 min, then air dry 5 min</li> <li>3) Circle tissue with a hydrophobic barrier pen</li> </ol>
<b>2</b>	<b>Pre-Treatment 1</b>	<b>Pretreat 1</b> (~8 drops/slide) 10 min @ RT, then rinse 1 X in <b>dH<sub>2</sub>O</b>
<b>3</b>	<b>Pre-Treatment 2</b>	Boil slides in 1X <b>Pretreat 2</b> 15 min, then rinse 2 X in <b>dH<sub>2</sub>O</b>
<b>4</b>	<b>Pre-Treatment 3 *</b>	<b>Pretreat 3</b> (~4 drops/slide) 30 min @ 40 °C, then rinse 2 X in <b>dH<sub>2</sub>O</b>
<b>5</b>	<b>Target Hybridization</b>	<b>Probe</b> (~4 drops/slide) 2 hrs @ 40 °C, then 2 X 2 min in <b>Wash Buffer</b>
<b>6</b>	<b>Amplification 1</b>	<b>Amp 1</b> (~4 drops/slide) 30 min @ 40 °C, then 2 X 2 min in <b>Wash Buffer</b>
<b>7</b>	<b>Amplification 2</b>	<b>Amp 2</b> (~4 drops/slide) 15 min @ 40 °C, then 2 X 2 min in <b>Wash Buffer</b>
<b>8</b>	<b>Amplification 3</b>	<b>Amp 3</b> (~4 drops/slide) 30 min @ 40 °C, then 2 X 2 min in <b>Wash Buffer</b>
<b>9</b>	<b>Amplification 4</b>	<b>Amp 4</b> (~4 drops/slide) 15 min @ 40 °C, then 2 X 2 min in <b>Wash Buffer</b>
<b>10</b>	<b>Detection</b>	Mix <b>DAB-A, B</b> @ 1:1, add 120 uL/slide, 10 min @ RT, then 1X in <b>dH<sub>2</sub>O</b>
<b>11</b>	<b>Counterstain &amp; Mounting</b>	<ol style="list-style-type: none"> <li>1) Gill's <b>Hematoxylin</b> (diluted 1:4 in dH<sub>2</sub>O) 3 min</li> <li>2) ~5 dips in ammonia water, then ~5 dips in <b>dH<sub>2</sub>O</b></li> <li>3) Dehydrate 1X 2 min in 50%, 70% and 100% <b>ethanol</b></li> <li>4) <b>Xylene</b> 5 min</li> <li>5) Mount with xylene-based mounting media</li> </ol>

\* Critical protease digestion step which may require further optimization.

Watch the demo video at [http://www.acdbio.com/demo\\_video.html](http://www.acdbio.com/demo_video.html)