

## Section IV: Tissue Specimen Preparation

<b>Overview</b>	The tissue specimen preparation is the same for both RNAscope <sup>®</sup> 1.0 and 2.0 FFPE Assays. Both have been designed to work with sectioned FFPE tissue specimens affixed to glass slides.
<b>Tissue Types</b>	<b>Tissue derived from cancers of breast, colon, lung, and prostate</b> were used to optimize the RNAscope protocol. Assay condition for other tissue types may require some optimization of <b>Pretreatment 2 and 3</b> as described in <a href="#">Section V: Assay Optimization Guideline</a> and further in <a href="#">Section IX: Tissue Specimen Evaluation</a> .
<b>FFPE Tissue Block Preparation</b> <ul style="list-style-type: none"><li>• Tissue: cut to 3-4 mm in thickness</li><li>• Fixative: 10% neutral-buffered formalin</li><li>• Fixation time: 16 – 32 hrs</li><li>• Fixation temperature: room temp</li></ul>	<b>Proper tissue preparation requires specimen to be cut into 3-4 mm in thickness and fixed in fresh 10% neutral-buffered formalin for 16-32 hours at room temperature (25°C) and embedding in paraffin.</b> <p>RNAscope is virtually certain to be successful with tissues that are properly fixed and embedded within three (3) years of use.</p> <hr/> <b><i>NOTE: Under-fixation will result in significant loss of signal and weak RNA staining.</i></b> <hr/>
<b>Tissue Slide Preparation</b> <ul style="list-style-type: none"><li>• Section: 5 +/- 1 µm</li><li>• Bake slides: 1 hr, 60°C</li><li>• Store: &lt; 1 week (desiccated)</li></ul>	Tissue specimens should be cut into sections of 5 +/- 1µm and mounted on SuperFrost Plus slides. <p>Tissue sections need to be air dried and baked in a dry oven at 60°C for 1 hour prior to the RNAscope<sup>®</sup> FFPE assay.</p> <p>Baked sections can be used in RNAscope immediately or can be stored at room temperature (20 – 25 °C) with desiccants for up to one week.</p>
<b>Tissue Quality Assurance</b>	<ul style="list-style-type: none"><li>• <b>FFPE tissue sample quality control is essential, particularly for retrospective specimens.</b></li><li>• It is recommended to perform Pretreatment Optimization for all new tissue types used, or if you are a first time user of RNAscope.</li><li>• For any new FFPE sample types or previously untested FFPE tissues, first run positive (UBC or POLR2A) and negative (dapB) control probes only on representative samples to ensure proper FFPE tissue preparation and optimal permeabilization.</li></ul>
<b>Non-conforming Tissue Specimens</b>	Many tissue specimens prepared differently from what is described above may still work well with RNAscope <sup>®</sup> FFPE Assay as long as the pretreatment protocol is optimized. For such specimens please refer to <a href="#">Section V: Assay Optimization Guidelines</a> and <a href="#">Section IX: Tissue Specimen Evaluation</a> .