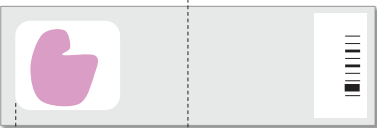


Section IV: Tissue Specimen Preparation

Overview	The tissue specimen preparation for RNAscopeVS FFPE Assay has been designed to work with sectioned FFPE tissue specimens affixed to glass slides.
Tissue Types	Tissue derived from cancers of breast, lung, and prostate were used to optimize the RNAscopeVS protocol. Assay condition for other tissue types may require some optimization of Pretreatment A and B steps as described in Section V: Assay Optimization Guideline and further in Section IX: Tissue Specimen Evaluation .
FFPE Tissue Block Preparation	<ul style="list-style-type: none">• Cut tissue specimen into blocks of 3 – 4 mm in thickness;• Fix tissue blocks in fresh 10% neutral-buffered formalin for 16-32 hours at room temperature (25 °C);• Embed fixed tissue blocks in paraffin. <p>NOTE: <i>Under-fixation will result in significant loss of signal and weak RNA staining.</i></p>
Tissue Slide Preparation	<ul style="list-style-type: none">• Cut tissue block into sections of 5 +/- 1µm in thickness;• Mount tissue section on SuperFrost Plus slides. Make sure to place the tissue section closer to the bottom end of the slide by aligning one edge of the paraffin close to the bottom edge of the slide (see below) <div data-bbox="743 1150 1117 1312" style="text-align: center;"><p>← Align one edge of the paraffin close to the bottom edge of the slide (away from barcode)</p></div> <ul style="list-style-type: none">• Air dry the mounted tissue sections and bake the air dried slide in a dry oven at 60 °C for 1 hour prior to the RNAscopeVS FFPE assay• Option online baking step can be added to the automated protocol if run the assay overnight.• Baked sections can be used in RNAscope assay immediately or can be stored at room temperature (20 – 25 °C) with desiccants for up to one week.
Tissue Quality Assurance	<ul style="list-style-type: none">• FFPE tissue sample quality control is essential, particularly for retrospective specimens.• It is recommended to perform Pretreatment Optimization for all new tissue types used, or if you are a first time user of RNAscope.• For any new FFPE sample types or previously untested FFPE tissues, first run positive (PPIB) and negative (dapB) control probes only on representative samples to ensure optimal pretreatment conditions for the FFPE tissues.

**Non-conforming
Tissue Specimens**

Many tissue specimens prepared differently from what is described above may still work well with RNAscopeVS FFPE Assay as long as the pretreatment protocol is optimized. For such specimens please refer to [Section V: Assay Optimization Guidelines](#) and [Section IX: Tissue Specimen Evaluation](#).