

# RNAscope® 2.5 LSx Reagent Kit (BROWN) Quick Guide

For FFPE Tissues

## Introduction

This quick guide is intended for advanced users who are familiar with the procedures in the *RNAscope® 2.5 LSx Reagent Kit-BROWN User Manual* (Document No. 322700-USM). 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: Reagents and Software

Workflow Steps	
MATERIALS FROM ACD	<ul style="list-style-type: none"> <li>The RNAscope® 2.5 LSx Reagent Kit – BROWN (Cat. No. 322700) consisting of two trays of reagents in labeled Leica BOND compatible containers               <ul style="list-style-type: none"> <li>The first tray has two tray barcodes and a label on the handle, and contains the LS Rinse, DAB chromogen, and counterstaining reagents</li> <li>The second tray contains the pretreatment and signal amplification reagents</li> </ul> </li> <li>RNAscope® 2.5 LS control probes and custom probes</li> </ul>
MATERIALS FROM LEICA	<ul style="list-style-type: none"> <li>Leica Biosystems' BOND RX System — automated slide stainer</li> <li>BOND Open Containers 30 mL</li> <li>BOND Universal Covertiles 100 pack</li> <li>BOND bulk buffers, including ER buffers, wash buffer, and dewax buffer</li> </ul>
VERIFY THE SOFTWARE VERSION	<ol style="list-style-type: none"> <li>Update the BDZ/BXD software to database version 1.5 before running the RNAscope® 2.5 LSx Brown Assay.               <p><b>Note:</b> Contact your ACD FAS for help with the 5.2 software database upgrade. To upgrade 4.0 software, contact a Leica field service scientist.</p> <p><b>Note:</b> The LSx assay does not need a workaround protocol or mock probe. Each probe is used as a marker.</p> </li> </ol>

## Part 2: Set up RNAscope® 2.5 LSx Assay

Workflow Steps	
REGISTER THE LSx REAGENTS ~5–10 MIN	<ol style="list-style-type: none"> <li>Register the reagents on the first tray by scanning the two tray barcodes sequentially with the Barcode Scanner. Select <b>OK</b>.</li> <li>Register each component separately on the second tray by scanning the barcode on each reagent container with the Barcode Scanner. Select <b>OK</b>.</li> </ol>
REGISTER PROBES ~10–20 MIN	Register each probe as a marker for the ISH procedure: <ol style="list-style-type: none"> <li>Go to the <b>Reagent Setup</b> screen, and select <b>Add</b> to enter the probe name, type, and other information.</li> </ol>

Workflow Steps	
	<ol style="list-style-type: none"> <li>For software version 4.0, select <b>Probe</b> in the Type drop down menu and <b>RNA</b> for Probe Type. For software version 5.2, select <b>Probe RNA</b> in the Type drop down menu.</li> <li>Select the default protocols you want to use for the probe.</li> <li>Select <b>Hazardous</b>, then <b>Save</b>.</li> </ol>
<p>SET UP A STUDY ~10–30 MIN</p>	<ol style="list-style-type: none"> <li>Go to the <b>Slide setup</b> screen, select <b>Add Study</b>, and enter information.</li> <li>For each slide and probe used in the assay:               <ol style="list-style-type: none"> <li>Go to the <b>Add slide</b> screen, select <b>ISH</b> and the probe of interest under the Marker drop down menu.</li> <li>In the Protocols tab, choose the following LSx protocols. Do not use protocols from other LS assays.                   <ol style="list-style-type: none"> <li>Staining: RNAscope 2.5 LSx DAB ISH</li> <li>HIER: RNAscope 2.5 LSx Target Retrieval (95/88)</li> <li>Enzyme: RNAscope 2.5 LSx Enzyme</li> <li>Hybridization: RNAscope 2.5 LSx Hybridization</li> </ol> </li> <li>Select <b>Add slide</b> to add each slide, and then select <b>Close</b>.</li> </ol> </li> <li>Select <b>Print labels</b> to print barcodes to attach to the slides.</li> </ol>
<p>START THE ASSAY ~10–30 MIN</p>	<ol style="list-style-type: none"> <li>Prepare the instrument, including filling the bulk reagents and emptying the waste container.</li> <li>Load the two registered 2.5 LSx reagent trays onto the instrument.</li> <li>Empty each target probe into a new 30mL Open container. Register each probe container by scanning the barcode in the front and selecting the corresponding name of the probe. Load probes onto the instrument.</li> <li>Go to the <b>Slide setup</b> screen.</li> <li>Attach the barcodes to the slides, add the covertiles, and place the slides on the slide tray with the label sides facing up.</li> <li>Place the tray in the Leica BOND RX, and press the button to load the tray onto the machine.</li> <li>After scanning the slides, select the PLAY (triangular) button on the screen located under the start tray. You may also right-click on the scanned label images, and select <b>Delayed Start</b> to start the run at a future time.</li> </ol>

## Troubleshooting

For troubleshooting information, please contact technical support at [support.acd@bio-techne.com](mailto:support.acd@bio-techne.com).

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### Headquarters

7707 Gateway Blvd Suite 200, Newark, CA 94545 Phone 1-510-576-8800 Toll Free 1-877-576-3636

For support, email [support.acd@bio-techne.com](mailto:support.acd@bio-techne.com)

[www.acdbio.com](http://www.acdbio.com)

