

RNAscope® 2.5 LSx Reagent Kit (RED) 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-RED User Manual* (Document No. 322750-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"> The RNAscope® 2.5 LSx Reagent Kit – RED (Cat. No. 322750), consisting of two trays of reagents in labeled Leica BOND compatible containers: <ul style="list-style-type: none"> The first tray has two tray barcodes and a label on the handle, and contains the LS Rinse, RED chromogen, and counterstaining reagents The second tray contains the pretreatment and signal amplification reagents RNAscope® 2.5 LS control probes and custom probes
MATERIALS FROM LEICA	<ul style="list-style-type: none"> Leica Biosystems' BOND RX System — automated slide stainer BOND Open Containers 30 mL BOND Universal Covertiles 100 pack BOND bulk buffers, including ER buffers, wash buffer, and dewax buffer
VERIFY THE SOFTWARE VERSION	<ol style="list-style-type: none"> Update the BDZ/BXD software to database version 1.5 before running the RNAscope® 2.5 LSx Red Assay. <p>Note: 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>Note: The LSx assay does not need a workaround protocol or mock probe. Each probe is used as a marker.</p>

Part 2: Set up RNAscope® 2.5 LSx Assay

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

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

Troubleshooting

For troubleshooting information, please contact technical support at support.acd@bio-techne.com.

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