

Using LS PretreatPro™ with LSx reagent kits

For use with Leica Biosystems' BOND RX System

Introduction

This quick guide is intended for users of the BROWN and Red RNAscope 2.5 LSx Reagent Kits (Product Numbers 322700 and 322750, respectively). This quick guide assumes users are familiar with the procedures in the *RNAscope™ 2.5 LS Reagent Kit User Guides* (UM 322100 and UM 322150) available on our website. Please refer to the user manuals for safety guidelines. Read the Safety Data Sheet (SDS) for every chemical 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: Prepare the instrument reagents

In this workflow, two new reagents are used in place of traditional reagents.

Traditional protease workflow	New protease-free workflow
RNAscope 2.5 LSx Protease	RNAscope 2.5 LS PretreatPro™
RNAscope 2.5 LSx AMP 5 BROWN/RED	RNAscope 2.5 LS AMP Pro™

1. Place RNAscope 2.5 LS PretreatPro™ into a BOND Open container and register it as *RNAscope 2.5 LSx Protease.

Note: Visually identify the container as LS PretreatPro to avoid unintended use.

2. Register RNAscope 2.5 LS AMP Pro as a new reagent (for example, ACD Amp Pro) in the BOND database. Please refer to Chapter 5 of the LS 2.5 Brown User Manual (Doc # UM 322100) for instructions on registering the reagents.
3. Place the *RNAscope 2.5 LS AMP Pro* reagent into a BOND Open container and register it as reagent created.

Note: When using these new reagents to stain samples, close the prefilled containers for *RNAscope 2.5 LSx Enzyme and *RNAscope 2.5 LSx AMP 5 (BROWN or RED).

4. Here is a summary of how containers will be registered before setting up a staining run. Most reagents auto-register when the kit is scanned into the database.

Reagents	Container Name
RNAscope 2.5 LS PretreatPro	*RNAscope 2.5 LSx Protease
RNAscope 2.5 LS AMP Pro	ACD Amp Pro (or appropriate)
RNAscope 2.5 LSx Hydrogen Peroxide	*RNAscope 2.5 LSx Hydrogen Peroxide
RNAscope 2.5 LSx Protease	*RNAscope 2.5 LSx Protease
RNAscope 2.5 LSx AMP 1 (BROWN or RED)	*RNAscope 2.5 LSx AMP 1 (BROWN or RED)
RNAscope 2.5 LSx AMP 2 (BROWN or RED)	*RNAscope 2.5 LSx AMP 2 (BROWN or RED)
RNAscope 2.5 LSx AMP 3 (BROWN or RED)	*RNAscope 2.5 LSx AMP 3 (BROWN or RED)
RNAscope 2.5 LS AMP 4 (BROWN or RED)	*RNAscope 2.5 LSx AMP 4 (BROWN or RED)
RNAscope 2.5 LSx AMP 5 (BROWN or RED)	*RNAscope 2.5 LSx AMP 5 (BROWN or RED)
RNAscope 2.5 LSx AMP 6 (BROWN or RED)	*RNAscope 2.5 LSx AMP 6 (BROWN or RED)
RNAscope 2.5 LSx Rinse	*RNAscope 2.5 LSx Rinse
RNAscope 2.5 LSx Bluing Reagent	* RNAscope 2.5 LSx Bluing
RNAscope 2.5 LS Target Probe	Variable

*Indicates reagent is hard coded in software by Leica Biosystems

5. In the respective fields, enter the RNAscope 2.5 LS Reagent Kit lot number and the expiration date. Select **OK**.

Update the protocol

1. Filter **Protocol Group** by **Prestaining**, and filter for **Protocol type** by **Enzyme Pretreatment**. Find existing protocol ***RNAscope 2.5 LSx Protease** that includes the two reagents ***RNAscope 2.5 LSx Protease** and ***RNAscope 2.5 LSx Hydrogen Peroxide**.
2. Copy and create a new protocol:
 - a. Change the ***RNAscope 2.5 LSx Protease** incubation time to **15 MIN** per step for two steps and the temperature to **40°C**.
 - b. Keep ***RNAscope 2.5 LSx Hydrogen Peroxide** incubation time at **10 MIN** and temperature at **Ambient**. Save the protocol with a new name, such as **RNAscope 2.5 LSx PretreatPro**.
3. Refer to **Appendix C** of (Doc # UM 322100) for detailed instructions on editing an Enzyme treatment protocol.
4. Filter **Protocol Group** by **Staining**.
5. Copy the appropriate ***RNAscope 2.5 LSx BROWN/RED ISH** protocol and replace the Amp 5 reagent steps with the newly created **ACD Amp Pro** reagent using the same incubation conditions.
6. Run all the other parts of the assay as usual and change only these two reagents.
7. When loading reagents on the BOND RX, ensure that the prefilled containers for ***RNAscope 2.5 LSx Enzyme** and ***RNAscope 2.5 LSx AMP 5 (BROWN or RED)** are closed.
8. The final Slide setup for an FFPE sample should look like the following, considering proper HIER condition choice.
 - a. Marker Selection: Choose the appropriate probe reagent for the staining run.
 - b. Protocol selections:
 - i. Staining: Choose the edited staining protocol with **ACD Amp Pro**
 - ii. Preparation: Select ***Bake and Dewax**.
 - iii. HIER: Choose ***RNAscope 2.5 LSx Target Retrieval (95)**.
 - iv. Enzyme: Choose the protocol created in step 2.

IMPORTANT! Do not introduce bubbles into the solutions by shaking the containers. To mix reagents, gently invert the containers several times. If bubbles are present, leave the containers out at room temperature until the bubbles dissipate.

- v. Probe Application: Select ***RNAscope 2.5 LSx Probe Application**.
 - vi. Denaturation: Select ***...**
 - vii. Hybridization: Select ***RNAscope 2.5 LSx Hybridization**
 - viii. Probe Removal: Select ***RNAscope 2.5 LSx Probe Removal**.
- c. Select **Add slide**.
9. After adding all the slides to the study, select **Close** to return to the Slide setup screen.
 10. Proceed to Run the RNAscope 2.5 LSx Assay

Optimization Note: For all protease-free workflows using LS PretreatPro and LS AMP Pro involving a low RNA expression and/or a dense tissue like the liver or spleen, we recommend boosting the ISH signal by increasing the antigen retrieval strength [duration, temperature].

Troubleshooting

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

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