

# BaseScope™ LS Detection Reagent Kit User Manual for BDZ 11 or higher

For use with Leica Biosystems' BOND RX System

Document Number 323600-USM



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# 1

## Chapter 1. Product Information



Before using this product, read and understand the information in **Appendix D. Safety** on page 39 in this document.

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**IMPORTANT!** We recommend reading the entire user manual before beginning any protocols.

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### About this guide

This user manual provides guidelines and protocols to use the BaseScope™ LS Reagent Kit with the Leica Biosystems' BOND RX Research Advanced Staining System. The BaseScope™ LS Assays are compatible with a variety of sample types.

### Product description

#### Background

The BaseScope™ LS Assay uses a novel and proprietary method of *in situ* hybridization (ISH) to visualize single RNA molecules for splice variants and short targets in samples mounted on slides. The BaseScope™ LS Assay does not require the RNA-free environment used for traditional ISH. The assay is based on ACD's patented signal amplification and background suppression technology. Compared with the RNAscope® 2.5 LS Assay, the BaseScope™ Assay incorporates additional signal amplification steps, which makes it possible to detect RNA splicing variants, point mutations, small insertions or deletions, and short RNA targets (50–300 nucleotides).

#### Overview

**Figure 1** on page 6 illustrates the BaseScope™ LS Assay procedure. You can complete the procedure on the Leica Biosystems' BOND RX System in ~12–13 hours. Start with properly prepared samples. The BOND RX pretreats samples and hybridizes RNA-specific probes to target RNA. Subsequent steps amplify the signal followed by hybridization to alkaline phosphatase (AP)-labeled probes and detection with a red chromogenic substrate. Each single RNA transcript appears as a distinct dot of chromogen precipitate visible when using a common brightfield microscope at 40X magnification.

**Figure 1. Procedure overview**



1: Tissue section	2: Hybridize to target RNA	3: Amplify signal	4: Image
Start with properly prepared sections and load slides onto the instrument. Pretreat tissue to allow access to target RNA.	Hybridize gene-specific probe pairs to the target mRNA.	Hybridize probe to a cascade of signal amplification molecules. Add <b>Fast Red</b> substrate to detect target RNA.	Visualize target RNA with a standard bright field microscope.

## Kit contents and storage

The BaseScope™ LS Assay requires the BaseScope™ LS Probes and the BaseScope™ LS Reagents, available from Advanced Cell Diagnostics.

### BaseScope™ LS Probes

The BaseScope™ LS Probes consist of the user-specified Target Probe and the Positive and Negative Control Probes. Visit <https://acdbio.com/products> to find a gene-specific Target Probe or appropriate Control Probes. Each probe is sufficient for staining ~30 standard slides. The probes have a shelf life of two years from the bulk manufacturing date when stored as indicated in the following table:

Target Probes					
<input checked="" type="checkbox"/>	Reagent	Cat. No.	Content	Quantity	Storage
	BaseScope™ LS Target Probe – [species]– [gene]	Various	Probe targeting specific RNA	16 mL x 1 bottle	2–8°C
Control Probes					
<input checked="" type="checkbox"/>	Reagent	Cat. No.	Content	Quantity	Storage
	BaseScope™ LS — Positive Control Probe – Human (Hs)-PPIB-3ZZ	701038	Probe targeting common housekeeping gene	16 mL x 1 bottle	2–8°C
	BaseScope™ LS — Positive Control Probe – Mouse (Mm)-Ppib-3ZZ	701078	Probe targeting common housekeeping gene	16 mL x 1 bottle	2–8°C
	BaseScope™ LS — Positive Control Probe – Human (Hs)-PPIB-1ZZ	701078	Probe targeting common housekeeping gene	16 mL x 1 bottle	2–8°C
	BaseScope™ LS — Positive Control Probe – Mouse (Mm)-Ppib-1ZZ	712358	Probe targeting common housekeeping gene	16 mL x 1 bottle	2–8°C
	BaseScope™ LS – Negative Control Probe- DapB-3ZZ	701018	Probe targeting bacterial gene dapB	16 mL x 1 bottle	2–8°C
	BaseScope™ LS – Negative Control Probe- DapB-1ZZ	701028	Probe targeting bacterial gene dapB	16 mL x 1 bottle	2–8°C

**IMPORTANT!** When running the BaseScope™ LS Assay, select the appropriate control probes corresponding to your target probe. We recommend 1ZZ control probes for 1ZZ target probes, and 3ZZ control probes for target probes 2ZZ and higher. If you have any questions, contact support at [support.acd@bio-techne.com](mailto:support.acd@bio-techne.com).

## BaseScope™ LS Detection Reagents

The BaseScope™ LS Reagent Kit (Cat. No. 323600) contains all the reagents needed to run the BaseScope™ LS Assay on the Leica Biosystems' BOND RX System, except for the RNA-specific probes. The kits provide enough reagents to stain ~60 standard slides.

The reagents are Ready-To-Use (RTU) and are stored as indicated in the following table:

BaseScope™ LS Reagent Kit (Cat. No. 323600)			
<input checked="" type="checkbox"/>	Reagent	Quantity	Storage
	RNAscope® 2.5 LS Hydrogen Peroxide	21 mL x 1 bottle	2–8°C
	RNAscope® 2.5 LS Protease III	21 mL x 1 bottle	2–8°C
	BaseScope™ LS AMP 1	21 mL x 1 bottle	2–8°C
	BaseScope™ LS AMP 2	21 mL x 1 bottle	2–8°C
	BaseScope™ LS AMP 3	21 mL x 1 bottle	2–8°C
	BaseScope™ LS AMP 4	21 mL x 1 bottle	2–8°C
	BaseScope™ LS AMP 5	21 mL x 1 bottle	2–8°C
	BaseScope™ LS AMP 6	21 mL x 1 bottle	2–8°C
	BaseScope™ LS AMP 7	21 mL x 1 bottle	2–8°C
	BaseScope™ LS AMP 8	21 mL x 1 bottle	2–8°C
	RNAscope® 2.5 LS Rinse	29 mL x 4 bottles	2–8°C
	RNAscope® 2.5 LS Bluing Reagent (OPTIONAL)	21 mL x 1 bottle	2–8°C

**IMPORTANT!** Use only BaseScope™ LS Probes. Although some BaseScope™ reagents share common names with RNAscope® reagents, we do not recommend interchanging kit components across these assays. You may negatively affect the number of slides you can run with each kit.

## Required materials from Leica BOND RX

The BaseScope™ LS Assay requires specific materials and equipment available *only* from Leica Biosystems.

<input checked="" type="checkbox"/>	Component	Cat. No.	Storage
	BOND Open Containers 30 mL	Op309700	Room temp (20–25°C)
	BOND Universal Covertiles 100 pack	S21.2001	Room temp (20–25°C)
	BOND Epitope Retrieval Solution 1-1L (RTU)	AR9961	2–8°C
	BOND Epitope Retrieval Solution 2-1L (RTU)	AR9640	2–8°C
	BOND Dewax Solution – 1L (RTU)	AR9222	2–8°C
	BOND Wash Solution 10X Concentrate – 1L	AR9590	2–8°C
	BOND Polymer Refine Red Detection plus Hematoxylin*	DS9390	2–8°C
	BOND Aspirating Probe Cleaning System	CS9100	2–8°C
	BOND Mixing Stations	S21.1971	Room temp (20–25°C)

\* Do not substitute with any other chromogen kit.

## Equipment

<input checked="" type="checkbox"/>	Component	Cat. No.
	Leica Biosystems' BOND RX System — automated slide stainer	—

## User-supplied materials

**IMPORTANT!** Do not substitute other materials for the SuperFrost® Plus Slides listed in the following table.

<input checked="" type="checkbox"/>	Description	Supplier	Cat. No.
	SuperFrost® Plus Slides (required)	Fisher Scientific	12-550-15
	95% Ethanol (EtOH)	American Master Tech Scientific/MLS*	ALREACS
	Xylene	Fisher Scientific/MLS	X3P-1GAL
	10% neutral-buffered formalin (NBF)	MLS	—
	Paraffin wax	MLS	—
	1X PBS	MLS	—
	Microtome	MLS	—
	Drying oven, capable of holding temperature at 60 +/- 1°C (optional)	MLS	—
	EcoMount OR Vectamount	Biocare Medical Vector Labs	EM897 H-5000
	Tissue-Tek® Vertical 24 Slide Rack	American Master Tech Scientific/MLS	LWS2124
	Tissue-Tek® Staining Dish (4 required)	American Master Tech Scientific/MLS	LWS20WH
	Tissue-Tek® Clearing Agent Dish, xylene resistant (2 required)	American Master Tech Scientific/MLS	LWS20GR
	Cover Glass 24 x 50 mm	Fisher Scientific/MLS	12-545-F
	Distilled water	MLS	—
	Fume hood	MLS	—

\* Major Laboratory Supplier in North America. For other regions, please check Catalog Numbers with your local lab supplier.

# 2

## Chapter 2. Before You Begin

Prior to running the BaseScope™ LS Assay on your samples for the first time, we recommend that you:

- Become familiar with Leica Biosystems' BOND RX Research Advanced Staining System. Refer to the *Leica Biosystems' BOND RX System Instructions for Use*.
- Run the assay on RNAscope® Control Slides (Cat. No. 310045 for Human HeLa Cell Pellet, and Cat. No. 310023 for Mouse 3T3 Cell Pellet) using the BaseScope™ LS Positive and Negative Control Probes.

### Important procedural guidelines

- Start with properly fixed and prepared sections. Refer to **Chapter 3. Prepare Your Samples** on page 10 for preparation of FFPE slides. For preparation of other sample types, contact [support.acd@bio-techne.com](mailto:support.acd@bio-techne.com).
- Regularly maintain and clean your automated staining instrument.
- Always run positive and negative control probes on your sample to assess sample RNA quality and optimal permeabilization.
- Do not substitute required materials. Assay has been validated with these materials only.
- Follow the protocol exactly for best results.
- Do not let your sections dry out during the procedure.
- Use good laboratory practices and follow all necessary safety procedures. Refer to **Appendix D. Safety** on page 39 for more information.

# 3

## Chapter 3. Prepare Your Samples

The following protocols describe formalin-fixed, paraffin-embedded (FFPE) sample preparation and pretreatment.

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**IMPORTANT!** We highly recommend following these guidelines. We cannot guarantee assay results with other preparation methods.

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### Prepare FFPE sections

#### Materials required

- 
- 10% neutral buffered formalin (NBF)
  - 1X PBS
  - Paraffin wax
  - 95% Ethanol (EtOH)
  - Xylene
  - Microtome
  - Water bath
  - SuperFrost® Plus slides
- 

#### Fix the sample

1. Immediately following dissection cut the tissue into blocks of 3–4 mm in thickness.
2. Place the tissue blocks into fixative within **1 HR** of biopsy.
3. Fix the tissue in 10% NBF for **16–32 HRS** at **ROOM TEMPERATURE (RT)**. Fixation time will vary depending on tissue type and size.



**CAUTION!** Handle biological specimens appropriately.

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**IMPORTANT!** Fixation for **<16 HRS** or **>32 HRS** will impair the performance of the BaseScope™ LS Assay.

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#### Dehydrate, embed, and cut the sample

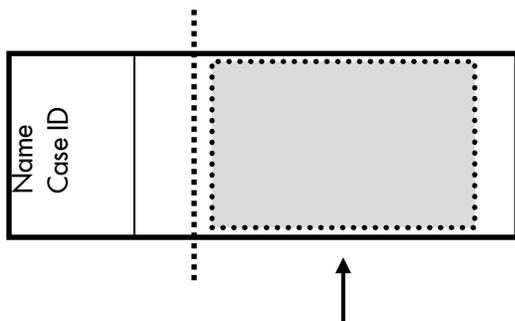
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**IMPORTANT!** Use fresh reagents.

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1. Wash sample with 1X PBS.
  2. Dehydrate sample using a standard ethanol series followed by xylene.
  3. Embed sample in paraffin using standard procedures.
- Note:** Embedded samples may be stored at room temperature with desiccation. To better preserve RNA quality over a long period (>1 yr), storing at 2–8°C with desiccation is recommended.
4. Trim paraffin blocks as needed, and cut the embedded tissue into  $5 \pm 1 \mu\text{m}$  sections using a microtome.

- Place the paraffin ribbon in a **40–45°C** water bath, and mount the sections on **SUPERFROST® PLUS SLIDES**. Place tissue as shown for optimal staining:



**Tissue section location**

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**IMPORTANT!** Do not mount more than one section per slide. Place each section in the center of the slide.

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- Air dry the slides **OVERNIGHT** at **RT**.

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**OPTIONAL STOPPING POINT** Use sectioned tissue within three months. Store sections with desiccants at room temperature.

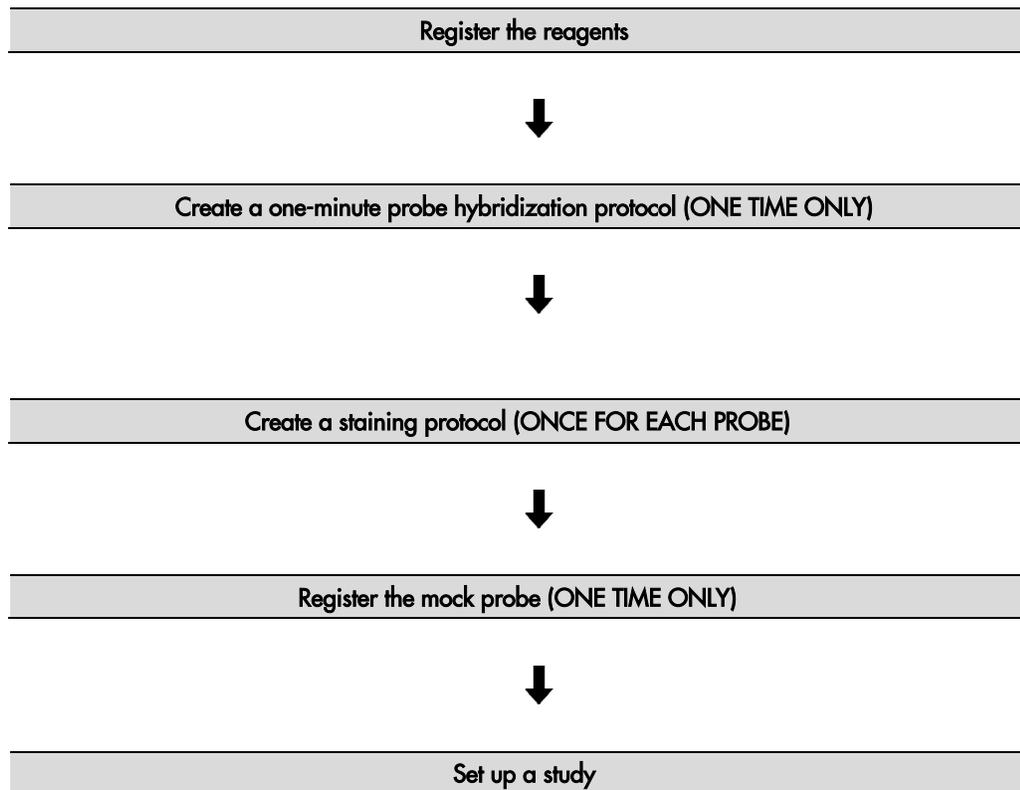
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# 4

## Chapter 4. Set Up the BDZ 11 Software

**IMPORTANT!** We strongly recommend you run the RNAscope® Control Slides (Cat. No. 310045 or Cat. No. 310023) using the BaseScope™ LS positive and negative control probes along with your samples in every run.

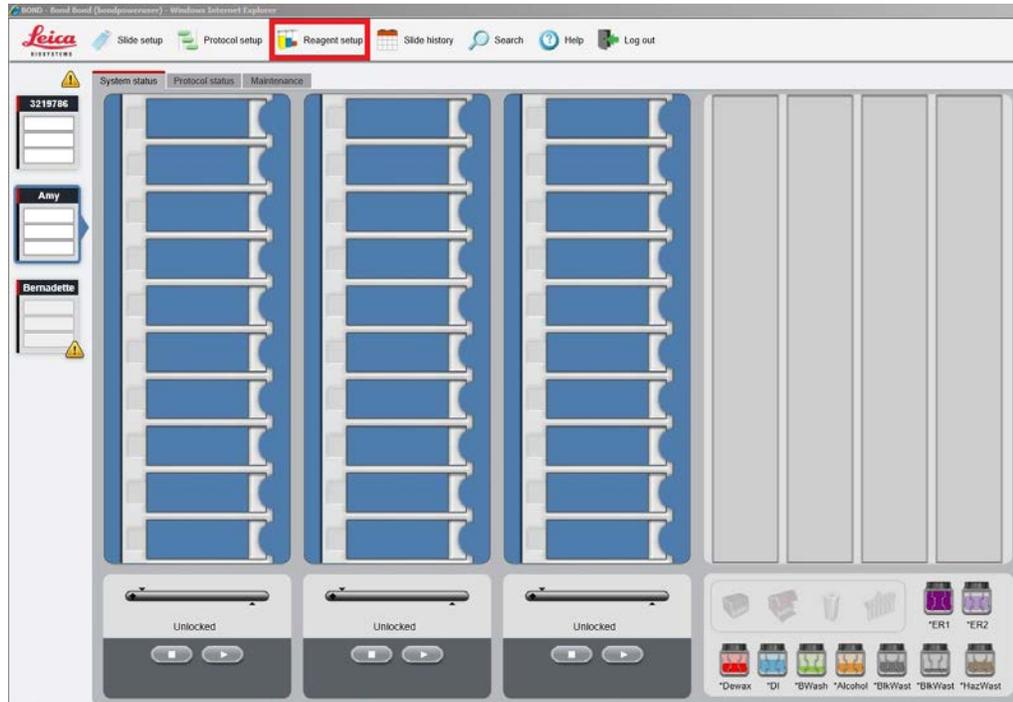
### Workflow



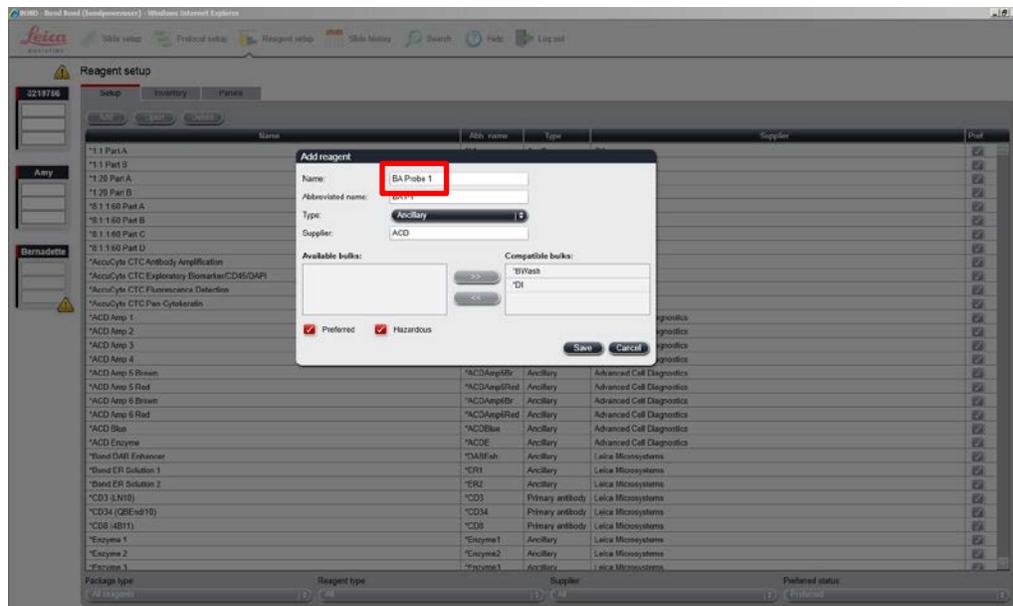
## Register the reagents

If you are using BDZ11 or a higher version of the database, you will need to add this “workaround” to accommodate the BaseScope™ LS Red assay. Your ACD Field Application Specialist (FAS) should implement this procedure. The workaround creates a probe as an ancillary reagent, which is added to the staining protocol.

1. Select the **Reagent Setup** icon at the top of the screen.



2. Select **Add** to enter reagent information.
3. To create a probe, enter a name (for example, **BA Probe 1**) in the Name text box.



4. Enter a name (for example, **BA P1** in the Abbreviated name text box.
5. Select **Ancillary** in the Type drop-down menu.

- Enter **ACD** in the Supplier text box.
- For the probe reagent only, check both the **Preferred** and **Hazardous** boxes.

**IMPORTANT!** Only probe reagents are marked Hazardous. BaseScope Amp reagents do not require that designation.

- Select **Save**.
- Repeat Steps 2–7 to register the rest of the reagents using the container names in the following table:

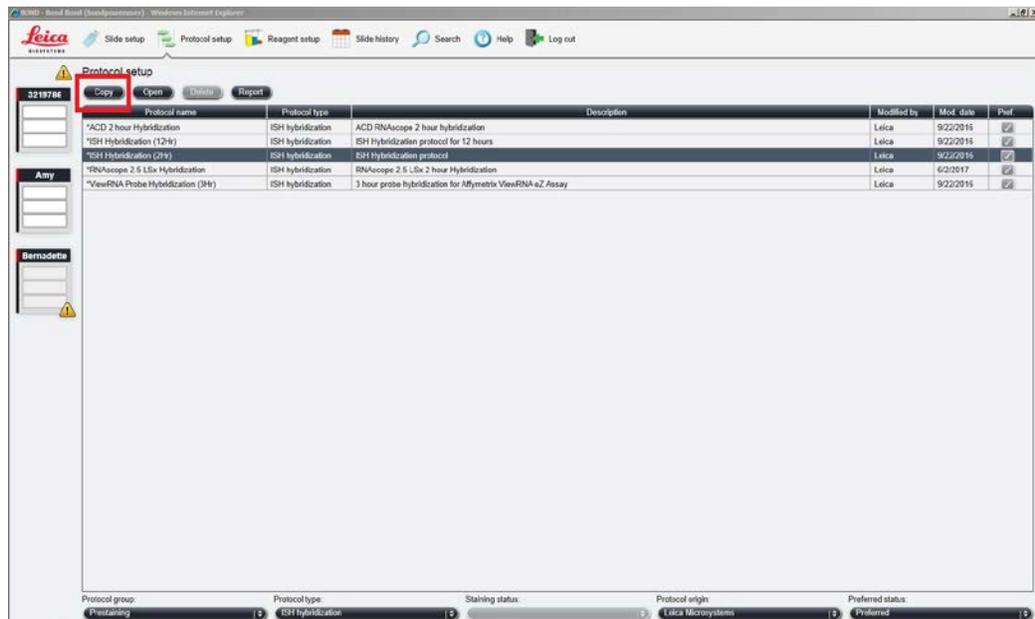
Reagents	Container Name	Abbreviation
BaseScope™ LS AMP 1	BaseScope Amp 1	BA Amp 1
BaseScope™ LS AMP 2	BaseScope Amp 2	BA Amp 2
BaseScope™ LS AMP 3	BaseScope Amp 3	BA Amp 3
BaseScope™ LS AMP 4	BaseScope Amp 4	BA Amp 4
BaseScope™ LS AMP 5	BaseScope Amp 5	BA Amp 5
BaseScope™ LS AMP 6	BaseScope Amp 6	BA Amp 6
BaseScope™ LS AMP 7	BaseScope Amp 7	BA Amp 7
BaseScope™ LS AMP 8	BaseScope Amp 8	BA Amp 8

**Note:** Perform these steps only once for each reagent and for each target probe.

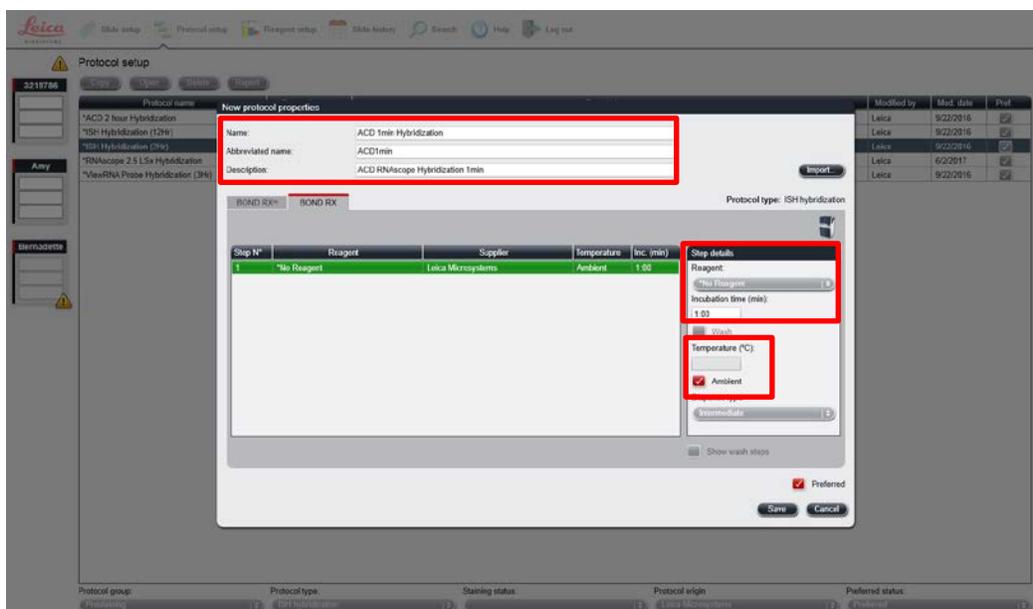
## Create a one-minute probe hybridization protocol

If you are using BDZ11 or higher version of the database, create a mock probe hybridization step as part of the workaround for the BaseScope™ LS assay. The following example copies the existing two-hour hybridization protocol and changes the incubation time to one minute.

- In the Protocol setup screen, select **ISH Hybridization** under the Protocol type menu.
- Highlight the **\*ISH Hybridization (2Hr)** protocol. Select **Copy**.



- Change the Name to **ACD 1min Hybridization**, the Abbreviated Name to **ACD1min**, and the Description to **ACD RNAscope hybridization 1min**.

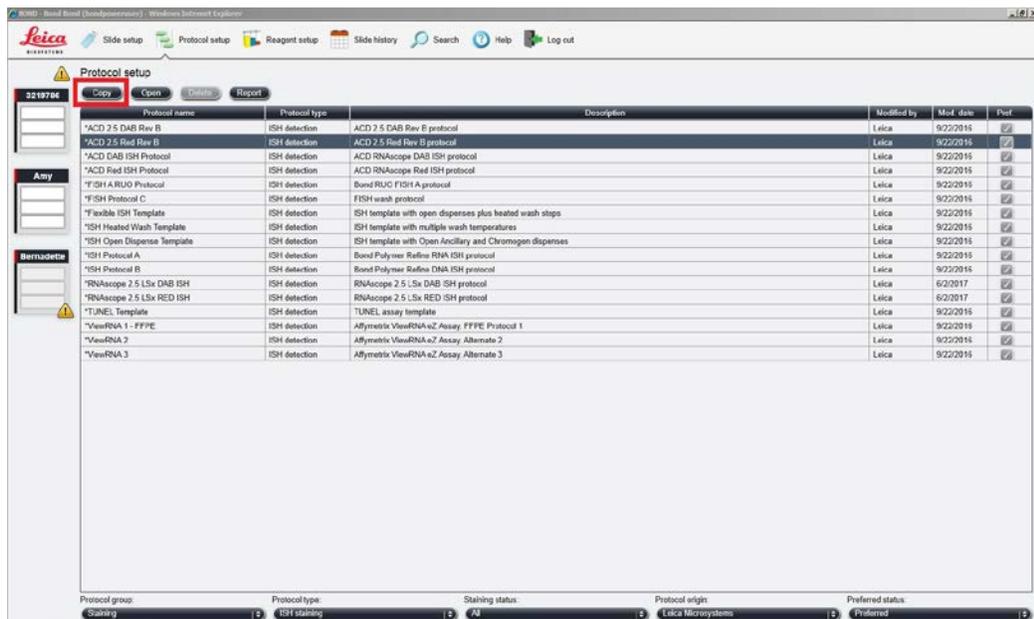


4. Highlight the **\*No Reagent** step.
5. Change the incubation time to **1 MIN** and select **Ambient** as Temperature (°C).
6. Select **Save**.

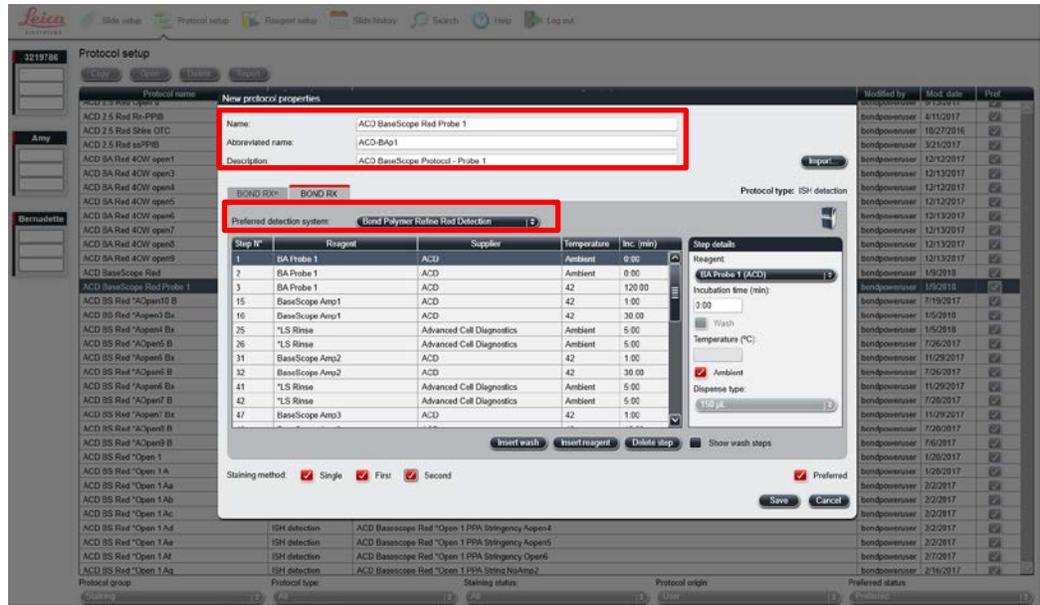
## Create staining protocols for each probe

To accommodate the BDZ11 or higher database version workaround, you must create unique staining protocols for each probe.

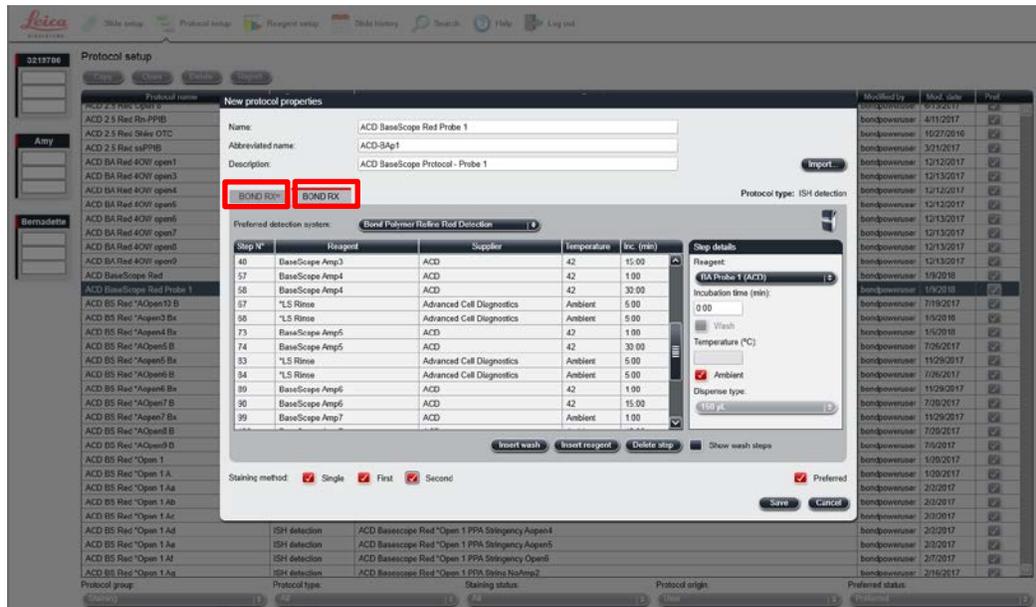
1. In the Protocol setup screen, select **Staining** under the Protocol group menu.
2. Highlight the **\*ACD 2.5 Red Rev B** protocol. Select **Copy**.

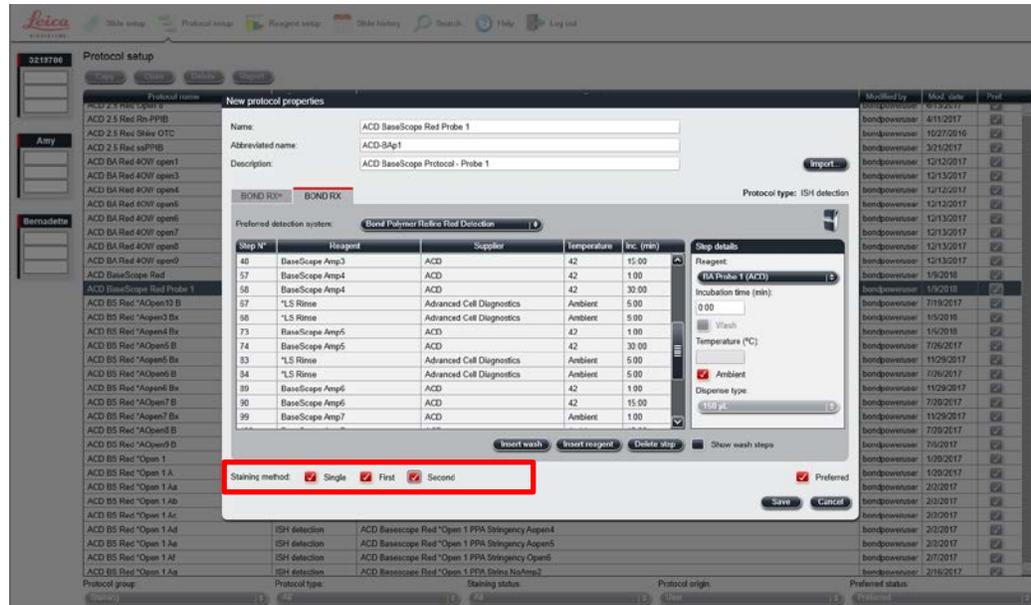


3. Change the protocol name for your first probe to **ACD BaseScope Red Probe 1** in the Name text box, **ACD-BAp1** in the Abbreviated name text box, and **ACD BaseScope Protocol – Probe 1** in the Description text box.
4. Confirm that **Bond Polymer Refine Red Detection** is selected for the Preferred detection system menu.



5. According to your specific instrument type, select either the BOND RX or BOND RXm tab.



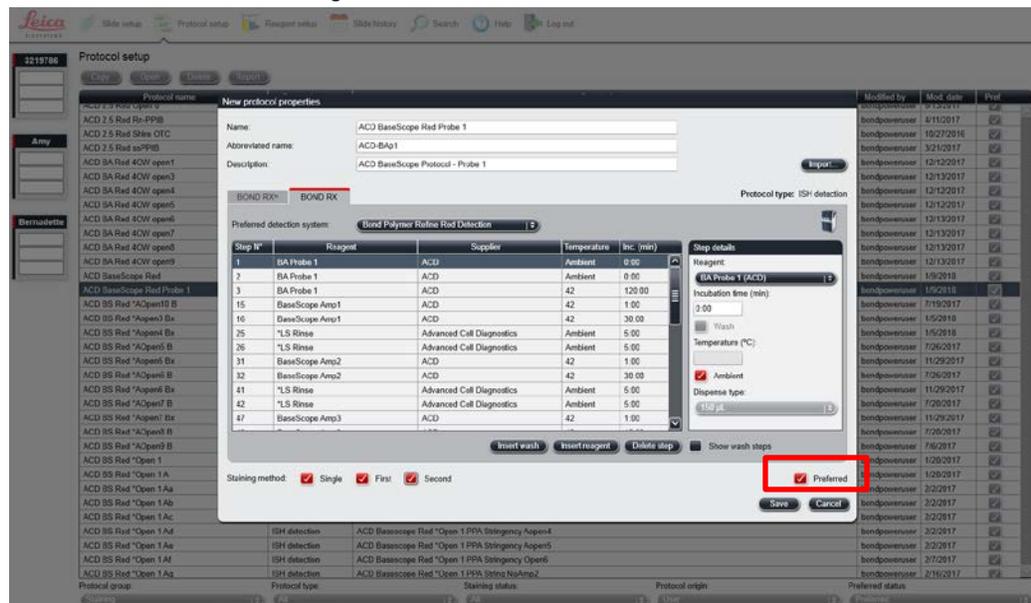


**Note:** The preceding three figures display all of the reagent steps.

**IMPORTANT!** You **must** select the **Single** button for the ISH protocol. Other buttons are optional.

**IMPORTANT!** If you are using the 4.0 software, you can only edit the incubation times of these steps. If you are using the 5.2 software, you can edit both times and temperatures.

6. Highlight and select each reagent step to edit it.
7. Click **Show wash steps** (see the following figure) to view the wash steps. Insert BOND Washes to match each of the protocol steps shown.
8. Compare and confirm the screen protocol with the full protocol listed in **Appendix A. Software Protocol** on page 29.
9. Select **Preferred** in the bottom right corner of the window.



10. Select **Save**.

11. Click **Next** to proceed. Ignore pop-ups that say Step 3 exceeds the recommended time and that the protocol is not validated by Leica. If you see any other pop-ups, please contact your FAS or technical support at ACD.

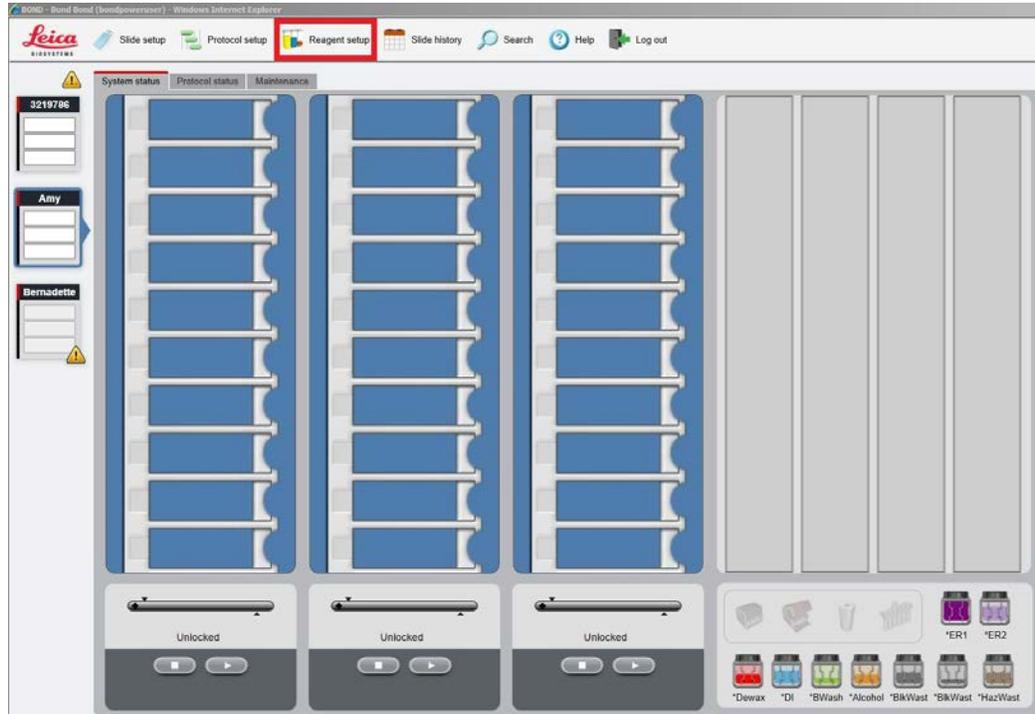
12. Create a new probe protocol.

**Note:** You must create a new protocol for each new probe you use.

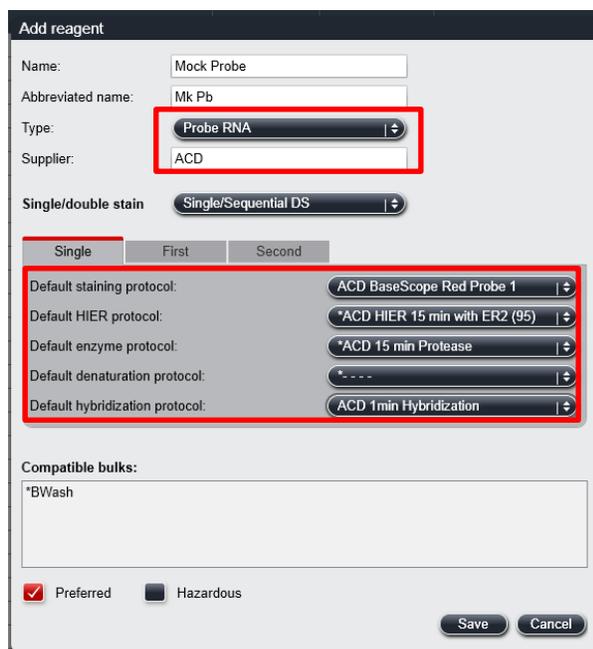
## Register the mock probe

Create a mock probe in the reagent set up.

1. Click the **Reagent setup** icon to register each probe.



2. Select **Add**.
3. Enter the **Mock Probe** in the Name and Abbreviated name text boxes.
4. Select **Probe RNA** in the Type drop-down menu. Enter **ACD** in the Supplier text box.



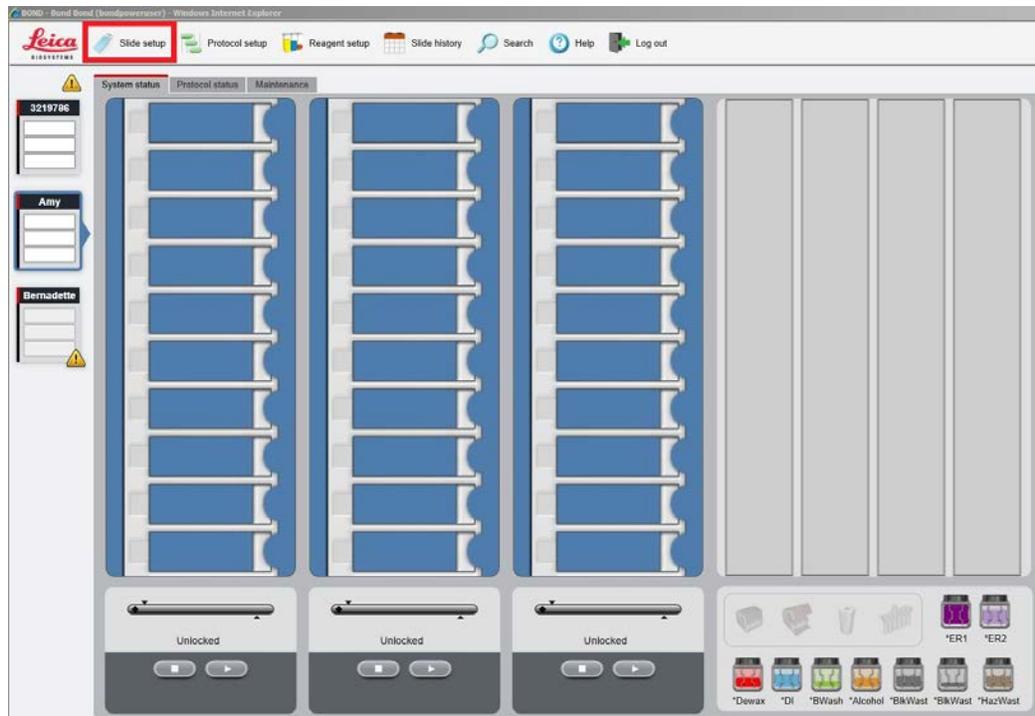
5. Select **ACD BaseScope Red Probe 1** as the Default staining protocol.
6. Select **\*ACD HIER 15min with ER2 (95)** as the Default HIER protocol.
7. Select **\*ACD 15min Protease** as the Default enzyme protocol.
8. Leave the Default denaturation protocol blank.
9. Select **ACD 1 min Hybridization** as the Default hybridization protocol.

**Note:** Mock Probe is not a hazardous reagent.

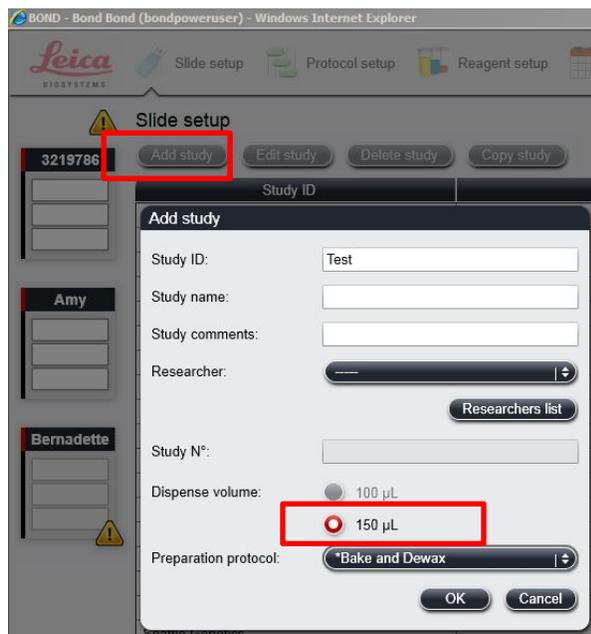
10. Select **Save**.

## Set up a study

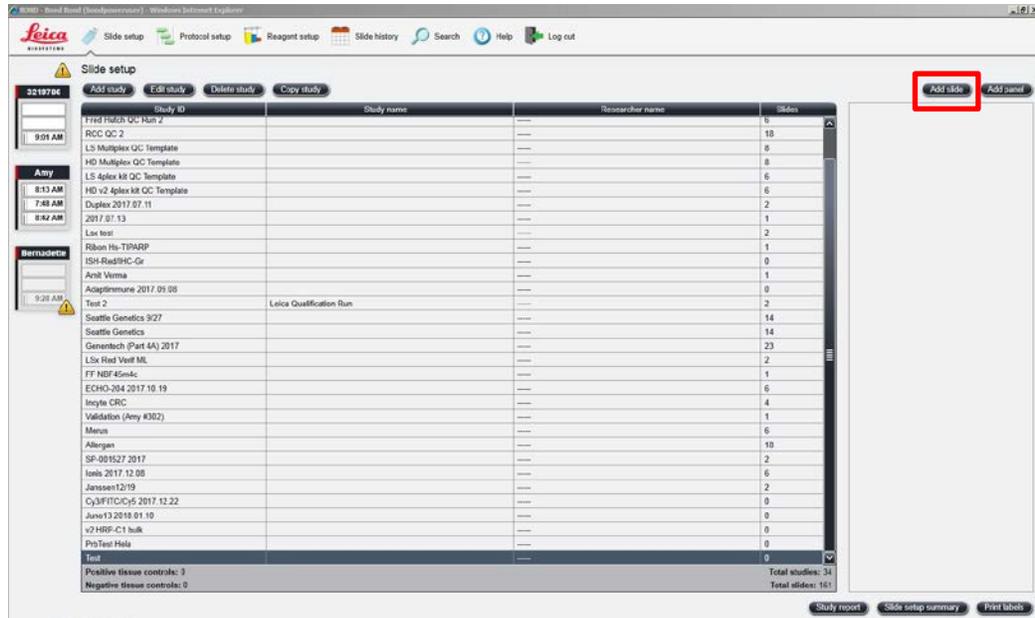
1. To build a study, select the **Slide setup** icon at the top of the screen.



2. Select **Add study** and enter a name in the **Study ID** field (keep the Dispense volume default as shown at **150**  $\mu\text{L}$ ).



3. Select **\*Bake and Dewax** as the Preparation protocol.
4. Select **OK**.
5. Select **Add slide** to assign a protocol to each slide.

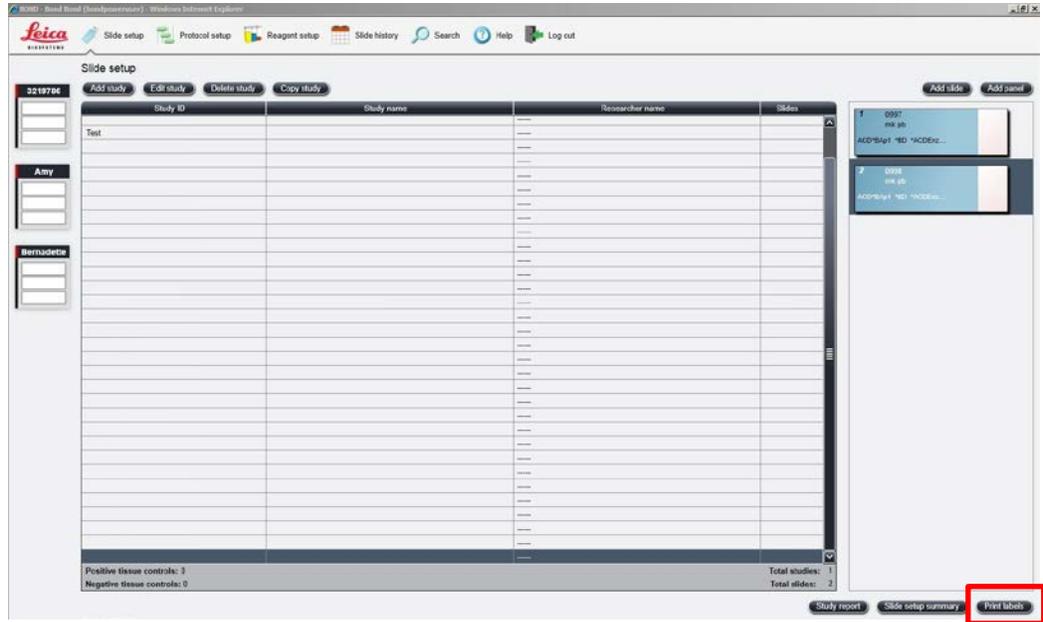


6. Enter the tissue type and probe name under the Comments field.

The 'Add slide' dialog box is shown. The 'Comments' field is highlighted with a red box and contains the text 'Tissue Type Probe 1'. The 'Process' dropdown is set to 'ISH' and the 'Marker' dropdown is set to 'Mock Probe (ACD)'. The 'Protocols' tab is selected, showing various staining and preparation options.

7. From the Research tab, select **ISH** under Process and **Mock Probe (ACD)** from the Marker drop down menu.
8. For BaseScope™ LS assays, under the **Protocols** tab:
  - a. Make sure that each probe is associated with a different protocol (for example, ACD BaseScope P1, P2), and select a protocol from the Staining drop down menu.
  - b. For standard FFPE tissues, select the protocol **\*Bake and Dewax** from the Preparation drop down menu.
  - c. Select **\*ACD HIER 15 min with ER2 (95)** as the HIER protocol.

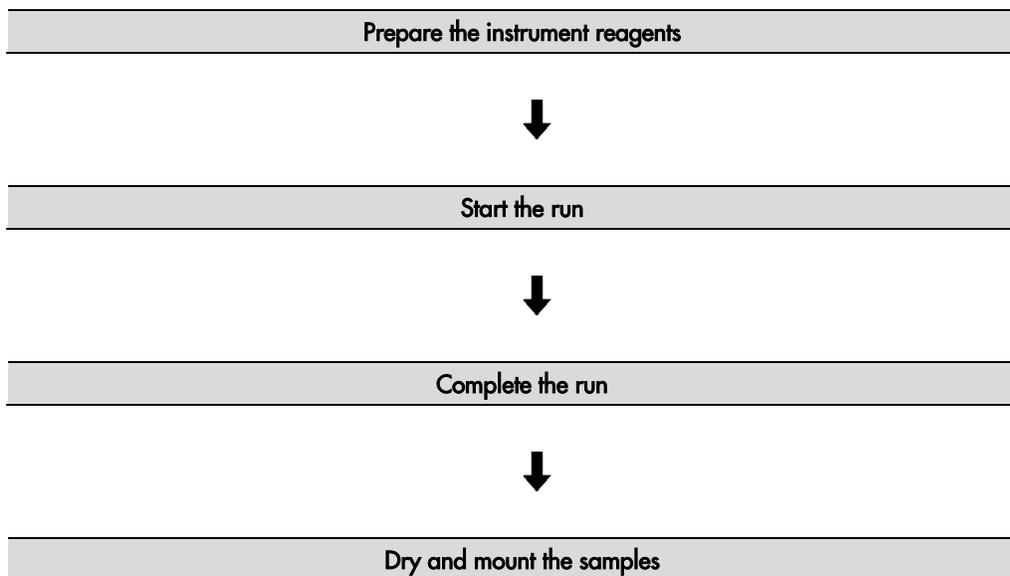
- d. Select **\*ACD HIER 15 min Protease** for Enzyme.
- e. Select **ACD 1 min Hybridization** for Hybridization.
9. Select **Add slide** for each target probe and for each of the slides used for the run.
10. After adding all the slides to the study, select **Close** to return to the Slide setup screen.
11. Select **Print labels** to print barcodes to attach to the slides.



# 5

## Chapter 5. Run the BaseScope™ LS Assay

### Workflow



## Materials required

Materials provided by Advanced Cell Diagnostics	Materials provided by Leica Biosystems	Materials provided by User
<ul style="list-style-type: none"> <li>• BaseScope™ LS Target Probe</li> <li>• BaseScope™ LS Positive Control Probe</li> <li>• BaseScope™ LS Negative Control Probe</li> <li>• RNAscope® 2.5 LS Hydrogen Peroxide</li> <li>• RNAscope® 2.5 LS Protease III</li> <li>• BaseScope™ LS AMP 1</li> <li>• BaseScope™ LS AMP 2</li> <li>• BaseScope™ LS AMP 3</li> <li>• BaseScope™ LS AMP 4</li> <li>• BaseScope™ LS AMP 5</li> <li>• BaseScope™ LS AMP 6</li> <li>• BaseScope™ LS AMP 7</li> <li>• BaseScope™ LS AMP 8</li> <li>• RNAscope® 2.5 LS Rinse</li> <li>• RNAscope® 2.5 LS Bluing Reagent (Optional)</li> </ul>	Leica Biosystems' BOND RX System <ul style="list-style-type: none"> <li>• Stainer</li> </ul> Bulk Reagents <ul style="list-style-type: none"> <li>• BOND Wash Solution, 10X</li> <li>• BOND Dewax Solution</li> <li>• BOND Epitope Retrieval Solution 1</li> <li>• BOND Epitope Retrieval Solution 2 Reagents</li> <li>• BOND Polymer Refine Red Detection plus Hematoxylin</li> </ul>	<ul style="list-style-type: none"> <li>• Distilled water</li> <li>• Drying oven</li> <li>• EcoMount</li> </ul>

## Prepare the instrument

1. Fill the large containers located in the bottom of the instrument with the Leica BOND RX bulk reagents. Dilute BOND Wash Solution 1:10.

**Note:** Insufficient bulk reagent volumes may lead to run failure.

**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.

2. Use clean, dry covertiles for every run. Following Leica's instructions, clean used covertiles with water, bleach, and ethanol. Air dry before reuse.
3. Before starting a run, empty bulk waste containers. Discard waste according to all local, state/provincial, and/or national regulations.

## Prepare the instrument reagents

1. Obtain one empty Open BOND container and label it "**Mock Probe**".
2. Fill the **Mock Probe** container with Leica Biosystems' 1X BOND Wash.
3. Carefully transfer all the BaseScope™ LS reagents into the empty 30 mL Open Bond containers.
4. Using the Barcode Scanner, scan the front barcode on the 30 mL Open Bond container. A window will appear.

**Note:** If using other open containers for probe delivery, ensure that you account for the dead-volume required in each container with volumes suggested in the following table.

Open Container	Suggested Dead-Volume
30 mL	2.5 mL
7 mL	1 mL
6 mL	600 µL

5. From the drop down menu, select the corresponding name of the reagent as shown in the following table under **Container Name**:

Reagents	Container Name
RNAscope® 2.5 LS Hydrogen Peroxide	*Open 0 Haz
RNAscope® 2.5 LS Protease III	*ACD Enzyme
BaseScope™ LS AMP 1	BaseScope Amp 1
BaseScope™ LS AMP2	BaseScope Amp 2
BaseScope™ LS AMP3	BaseScope Amp 3
BaseScope™ LS AMP4	BaseScope Amp 4
BaseScope™ LS AMP5	BaseScope Amp 5
BaseScope™ LS AMP6	BaseScope Amp 6
BaseScope™ LS AMP7	BaseScope Amp 7
BaseScope™ LS AMP8	BaseScope Amp 8
RNAscope® 2.5 LS Rinse	*LS Rinse
BaseScope™ LS Target Probe	Variable (BA Probe 1)
1X BOND Wash	Mock Probe
RNAscope® 2.5 LS Bluing Reagent (Optional)	*ACD Blue

\* Indicates reagent is hard-coded in software by Leica Biosystems.

**Note:** Leica BOND Red Detection plus Hematoxylin comes in a pre-filled Leica BOND RX container.

6. Enter the BaseScope™ LS Reagent Kit lot number and the expiration date in their respective fields. Select **OK**.

---

**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.

---

## Start the run

1. Attach the barcodes to the slides and add the slides to the slide tray with the label sides facing up.

**Note:** Add a covertile on top of each slide. The rectangular-shaped neck of the covertile should fit into the groove of the slide tray. Verify placement and seating of covertile.

2. Place the tray in the Leica BOND RX™, and press the button to load the tray onto the machine.
3. Once the slides have been scanned, select the **PLAY** (triangular) button on the screen located under the start tray to start the run. Alternatively, right-click on scanned label images and select **Delayed Start** to start the run at a future time.

---

**IMPORTANT!** Before leaving the instrument unattended, ensure that the instrument is running successfully. In the event of a problem, please refer to **Troubleshooting** on page 28.

---

## Complete the run

1. After the run is complete, press the button on the front of the instrument to unload the slides.
2. Place the slides onto the Tissue-Tek® Slide Rack and move the rack into a staining dish containing distilled water.
3. Flush the slides with distilled water for a few minutes.
4. (Optional) Perform offline bluing of choice, or use the RNAscope® 2.5 LS Bluing Reagent included in the kit by pipetting a small amount onto each slide for **1–2 MIN** followed by rinsing with distilled water.

## Dry and mount the samples

1. Remove the slide rack from the staining dish and dry slides in a **60°C** dry oven for **30 MIN**.

---

**IMPORTANT!** The RED substrate is alcohol sensitive. Do not dehydrate the slides in alcohol.

---

2. Cool the slides for **5 MIN** at **RT**.
3. Briefly dip one slide into fresh pure xylene and *immediately* place 1–2 drops of EcoMount or Vectamount on the slide before the xylene dries.

---

**IMPORTANT!** Use EcoMount (Biocare Medical) or Vectamount (VectorLabs).

---

4. Carefully place a 24 mm x 50 mm coverslip over the tissue section. Avoid trapping air bubbles.
5. Repeat steps 2 and 3 for each slide.
6. Air dry the slides for **5 MIN**.
7. Proceed to **Chapter 6. Evaluate the Results** on page 27.

# 6

## Chapter 6. Evaluate the Results

Examine tissue sections under a standard bright field microscope at 20–40X magnification:

- Assess tissue and cell morphology.
- Assess positive control signal strength. Positive control signal should be visible as punctate dots within the cell at 20–40X magnification.
- Assess negative control background. One dot to every 10 cells displaying background staining per 20X microscope field is acceptable.
- Evaluate target probe signal using the scoring guidelines in the next section.

### Scoring guidelines

The BaseScope™ LS Assay enables a semi-quantitative scoring guideline utilizing the estimated number of punctate dots present within each cell boundary.

An example of how to develop such a guideline for semi-quantitative assessment of RNAscope® staining intensity is presented below for a gene with expression level varying between 1 to > 10 copies per cell.

**Note:** If your gene expression level is higher or lower than this range, you may need to scale the criteria accordingly.

Categorize staining into five grades: **0**, **1+**, **2+**, **3+**, and **4+** according to the following table:

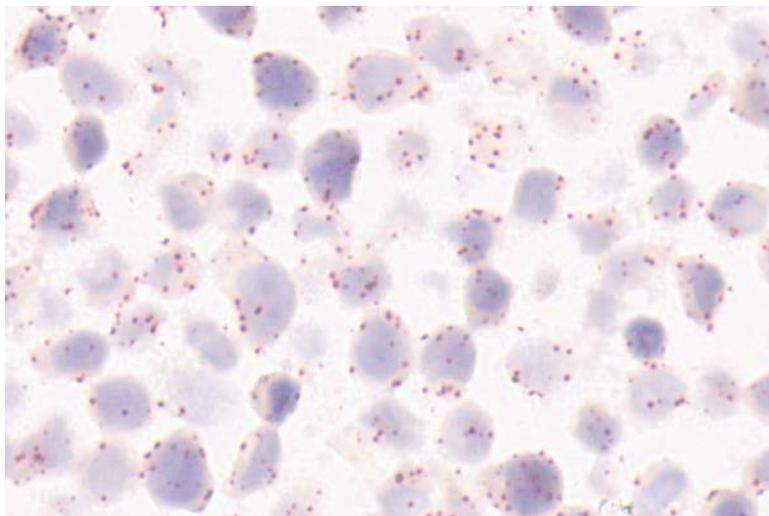
Staining Score	Microscope Objective Scoring*
0	No staining or less than 1 dot for every 20 cells (40X magnification)
1	1 dots/cell (visible at 20–40X magnification)
2	2–3 dots/cell. No or very few dot clusters (visible at 20–40X magnification)
3	4–10 dots/cell. Less than 10% positive cells have dot clusters (visible at 20X magnification)
4	>10 dots/cell. More than 10% positive cells have dot clusters (visible at 20X magnification)

\* Discount cells with artificially high nuclear background staining.

## Control example

If the assay is successful, the staining should look like the following image:

**Figure 2.** BaseScope™ LS Assay detection of POLR2A mRNA using Hs-POLR2A-1zz BaseScope™ LS Probe in HeLa cells



## Troubleshooting

If you obtain less than satisfactory results, troubleshoot your assay by following these simple guidelines:

- If you observe the presence of background staining, increase the Epitope Retrieval 2 (ER2) in increments of five minutes and increase the Enzyme time in increments of ten minutes (see **Appendix C. Edit the Protease Protocol** on page 37).
- The BaseScope™ LS assay utilizes Leica Biosystems' Bond Polymer Refine Red Detection kit. Do not use any other chromogen kits.
- Do not shake the contents in the dispensers as this will form bubbles and may lead to weak or no staining. If bubbles are present, leave the containers out at room temperature until the bubbles dissipate.
- Do not alter the staining protocol in any way with the exception of the hematoxylin incubation time. We have optimized the parameters in the staining protocol to run the BaseScope™ assay on the instrument.

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



# Appendix A. Software Protocol

The following table displays the full software protocol.

**Note:** Heated bond washes 4–6 come from the bulk reagents and are heated by the instrument. You cannot delete these steps. You may delete other wash steps.

**Note:** Occasionally, automated Bluing can cause a yellow precipitate in isolated tissues. You can eliminate this step if necessary.

Step No.	Reagent	Step Type	Incubation Time	Temperature
1	BA Probe 1	Reagent	0 MIN	Ambient
2	BA Probe 1	Reagent	0 MIN	Ambient
3	BA Probe 1	Reagent	120 MIN	42°C
4	*Bond Wash Solution	Wash	0 MIN	42°C
5	*Bond Wash Solution	Wash	1 MIN	42°C
6	*Bond Wash Solution	Wash	5 MIN	42°C
7	*Bond Wash Solution	Wash	0 MIN	Ambient
8	*Bond Wash Solution	Wash	0 MIN	Ambient
9	*Bond Wash Solution	Wash	0 MIN	Ambient
10	*Bond Wash Solution	Wash	0 MIN	Ambient
11	*Bond Wash Solution	Wash	0 MIN	Ambient
12	*Bond Wash Solution	Wash	1 MIN	Ambient
13	*Bond Wash Solution	Wash	1 MIN	Ambient
14	*Bond Wash Solution	Wash	0 MIN	Ambient
15	BaseScope Amp 1	Reagent	1 MIN	42°C
16	BaseScope Amp 1	Reagent	30 MIN	42°C
17	*Bond Wash Solution	Wash	0 MIN	Ambient
18	*Bond Wash Solution	Wash	0 MIN	Ambient
19	*Bond Wash Solution	Wash	0 MIN	Ambient
20	*Bond Wash Solution	Wash	3 MIN	Ambient
21	*Bond Wash Solution	Wash	3 MIN	Ambient
22	*Bond Wash Solution	Wash	0 MIN	Ambient
23	*Bond Wash Solution	Wash	0 MIN	Ambient
24	*Bond Wash Solution	Wash	0 MIN	Ambient
25	*LS Rinse	Reagent	5 MIN	Ambient
26	*LS Rinse	Reagent	5 MIN	Ambient
27	*Bond Wash Solution	Wash	0 MIN	Ambient

Step No.	Reagent	Step Type	Incubation Time	Temperature
28	*Bond Wash Solution	Wash	1 MIN	Ambient
29	*Bond Wash Solution	Open wash	1 MIN	Ambient
30	*Bond Wash Solution	Wash	1 MIN	Ambient
31	BaseScope Amp 2	Reagent	1 MIN	42°C
32	BaseScope Amp 2	Reagent	30 MIN	42°C
33	*Bond Wash Solution	Wash	0 MIN	Ambient
34	*Bond Wash Solution	Wash	0 MIN	Ambient
35	*Bond Wash Solution	Wash	0 MIN	Ambient
36	*Bond Wash Solution	Wash	3 MIN	Ambient
37	*Bond Wash Solution	Wash	3 MIN	Ambient
38	*Bond Wash Solution	Wash	0 MIN	Ambient
39	*Bond Wash Solution	Wash	0 MIN	Ambient
40	*Bond Wash Solution	Wash	0 MIN	Ambient
41	*LS Rinse	Reagent	5 MIN	Ambient
42	*LS Rinse	Reagent	5 MIN	Ambient
43	*Bond Wash Solution	Wash	0 MIN	Ambient
44	*Bond Wash Solution	Wash	1 MIN	Ambient
45	*Bond Wash Solution	Open wash	1 MIN	Ambient
46	*Bond Wash Solution	Wash	1 MIN	Ambient
47	BaseScope Amp 3	Reagent	1 MIN	42°C
48	BaseScope Amp 3	Reagent	15 MIN	42°C
49	*Bond Wash Solution	Wash	0 MIN	Ambient
50	*Bond Wash Solution	Wash	0 MIN	Ambient
51	*Bond Wash Solution	Wash	0 MIN	Ambient
52	*Bond Wash Solution	Wash	1 MIN	Ambient
53	*Bond Wash Solution	Wash	1 MIN	Ambient
54	*Bond Wash Solution	Wash	1 MIN	Ambient
55	*Bond Wash Solution	Wash	1 MIN	Ambient
56	*Bond Wash Solution	Wash	1 MIN	Ambient
57	BaseScope Amp 4	Reagent	1 MIN	42°C
58	BaseScope Amp 4	Reagent	30 MIN	42°C
59	*Bond Wash Solution	Wash	0 MIN	Ambient
60	*Bond Wash Solution	Wash	0 MIN	Ambient
61	*Bond Wash Solution	Wash	0 MIN	Ambient
62	*Bond Wash Solution	Wash	3 MIN	Ambient

Step No.	Reagent	Step Type	Incubation Time	Temperature
63	*Bond Wash Solution	Wash	3 MIN	Ambient
64	*Bond Wash Solution	Wash	0 MIN	Ambient
65	*Bond Wash Solution	Wash	0 MIN	Ambient
66	*Bond Wash Solution	Wash	0 MIN	Ambient
67	*LS Rinse	Reagent	5 MIN	Ambient
68	*LS Rinse	Reagent	5 MIN	Ambient
69	*Bond Wash Solution	Wash	0 MIN	Ambient
70	*Bond Wash Solution	Wash	1 MIN	Ambient
71	*Bond Wash Solution	Open wash	1 MIN	Ambient
72	*Bond Wash Solution	Wash	1 MIN	Ambient
73	BaseScope Amp 5	Reagent	1 MIN	42°C
74	BaseScope Amp 5	Reagent	30 MIN	42°C
75	*Bond Wash Solution	Wash	0 MIN	Ambient
76	*Bond Wash Solution	Wash	0 MIN	Ambient
77	*Bond Wash Solution	Wash	0 MIN	Ambient
78	*Bond Wash Solution	Wash	3 MIN	Ambient
79	*Bond Wash Solution	Wash	3 MIN	Ambient
80	*Bond Wash Solution	Wash	0 MIN	Ambient
81	*Bond Wash Solution	Wash	0 MIN	Ambient
82	*Bond Wash Solution	Wash	0 MIN	Ambient
83	*LS Rinse	Reagent	5 MIN	Ambient
84	*LS Rinse	Reagent	5 MIN	Ambient
85	*Bond Wash Solution	Wash	0 MIN	Ambient
86	*Bond Wash Solution	Wash	1 MIN	Ambient
87	*Bond Wash Solution	Wash	1 MIN	Ambient
88	*Bond Wash Solution	Wash	1 MIN	Ambient
89	BaseScope Amp 6	Reagent	1 MIN	42°C
90	BaseScope Amp 6	Reagent	15 MIN	42°C
91	*Bond Wash Solution	Wash	0 MIN	Ambient
92	*Bond Wash Solution	Wash	0 MIN	Ambient
93	*Bond Wash Solution	Wash	0 MIN	Ambient
94	*Bond Wash Solution	Wash	1 MIN	Ambient
95	*Bond Wash Solution	Wash	1 MIN	Ambient
96	*Bond Wash Solution	Wash	1 MIN	Ambient
97	*Bond Wash Solution	Open Wash	1 MIN	Ambient

Step No.	Reagent	Step Type	Incubation Time	Temperature
98	*Bond Wash Solution	Wash	1 MIN	Ambient
99	BaseScope Amp 7	Reagent	1 MIN	Ambient
100	BaseScope Amp 7	Reagent	15 MIN	Ambient
101	*Bond Wash Solution	Wash	0 MIN	Ambient
102	*Bond Wash Solution	Wash	0 MIN	Ambient
103	*Bond Wash Solution	Wash	0 MIN	Ambient
104	*Bond Wash Solution	Wash	1 MIN	Ambient
105	*Bond Wash Solution	Wash	1 MIN	Ambient
106	*Bond Wash Solution	Wash	1 MIN	Ambient
107	*Bond Wash Solution	Wash	1 MIN	Ambient
108	*Bond Wash Solution	Wash	1 MIN	Ambient
109	BaseScope Amp 8	Reagent	1 MIN	Ambient
110	BaseScope Amp 8	Reagent	15 MIN	Ambient
111	*Bond Wash Solution	Wash	0 MIN	Ambient
112	*Bond Wash Solution	Wash	0 MIN	Ambient
113	*Bond Wash Solution	Wash	0 MIN	Ambient
114	*Bond Wash Solution	Wash	1 MIN	Ambient
115	*Bond Wash Solution	Wash	1 MIN	Ambient
116	*Bond Wash Solution	Wash	1 MIN	Ambient
117	*Bond Wash Solution	Wash	1 MIN	Ambient
118	*Bond Wash Solution	Wash	1 MIN	Ambient
119	*LS Rinse	Reagent	5 MIN	Ambient
120	*LS Rinse	Reagent	5 MIN	Ambient
121	*Mixed RED Refine	Reagent	1 MIN	Ambient
122	*Mixed RED Refine	Reagent	10 MIN	Ambient
123	*De-ionized Water	Wash	0 MIN	Ambient
124	*De-ionized Water	Wash	0 MIN	Ambient
125	*De-ionized Water	Wash	0 MIN	Ambient
126	*De-ionized Water	Wash	0 MIN	Ambient
127	*De-ionized Water	Wash	0 MIN	Ambient
128	*Hematoxylin	Reagent	5 MIN	Ambient
129	*De-ionized Water	Wash	0 MIN	Ambient
130	*De-ionized Water	Wash	0 MIN	Ambient
131	*De-ionized Water	Wash	0 MIN	Ambient
132	*De-ionized Water	Wash	0 MIN	Ambient

Step No.	Reagent	Step Type	Incubation Time	Temperature
133	*ACD Blue (Optional)	Reagent	2 MIN	Ambient
134	*De-ionized Water (Optional)	Wash	0 MIN	Ambient
135	*De-ionized Water (Optional)	Wash	0 MIN	Ambient
136	*De-ionized Water (Optional)	Wash	0 MIN	Ambient
137	*De-ionized Water (Optional)	Wash	0 MIN	Ambient

\* Indicates reagent is hard-coded in software by Leica Biosystems.

---

**IMPORTANT!** If you are using the 4.0 software, you can only change the incubation times of these steps. If you are using the 5.2 software, you can change both the temperatures and incubation times.

---

**IMPORTANT!** Staining protocols longer than ~140 steps will limit the number of slide trays/SSA that can be run at one time.

---

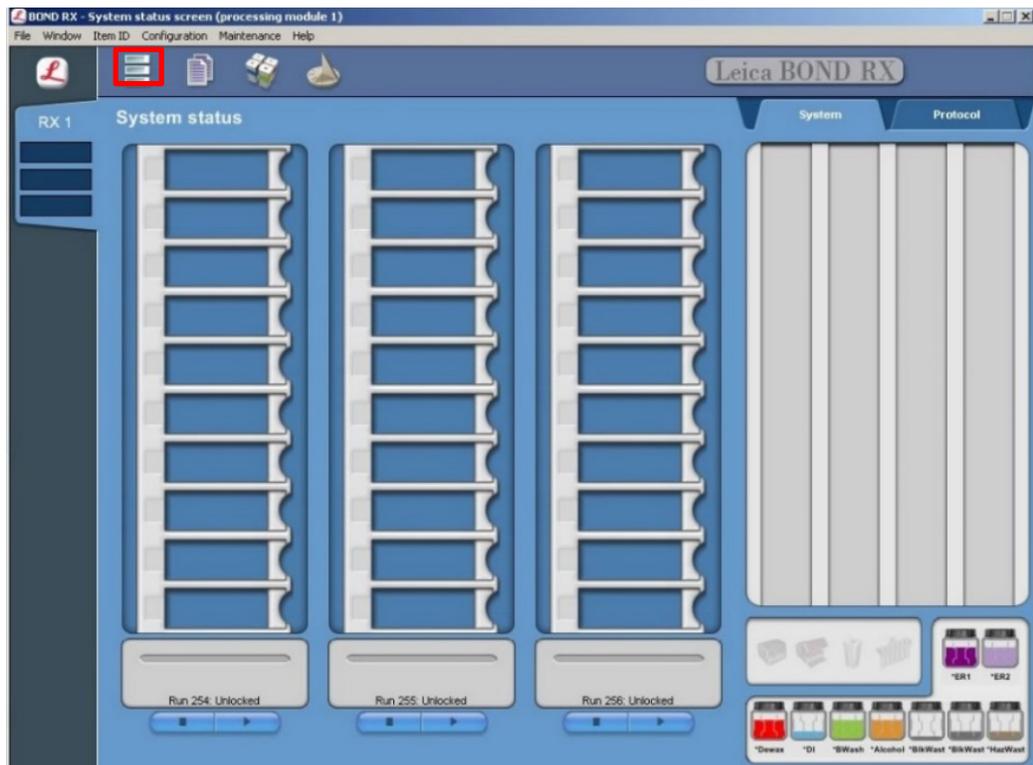
# B

## Appendix B. Edit the Epitope Retrieval Protocol

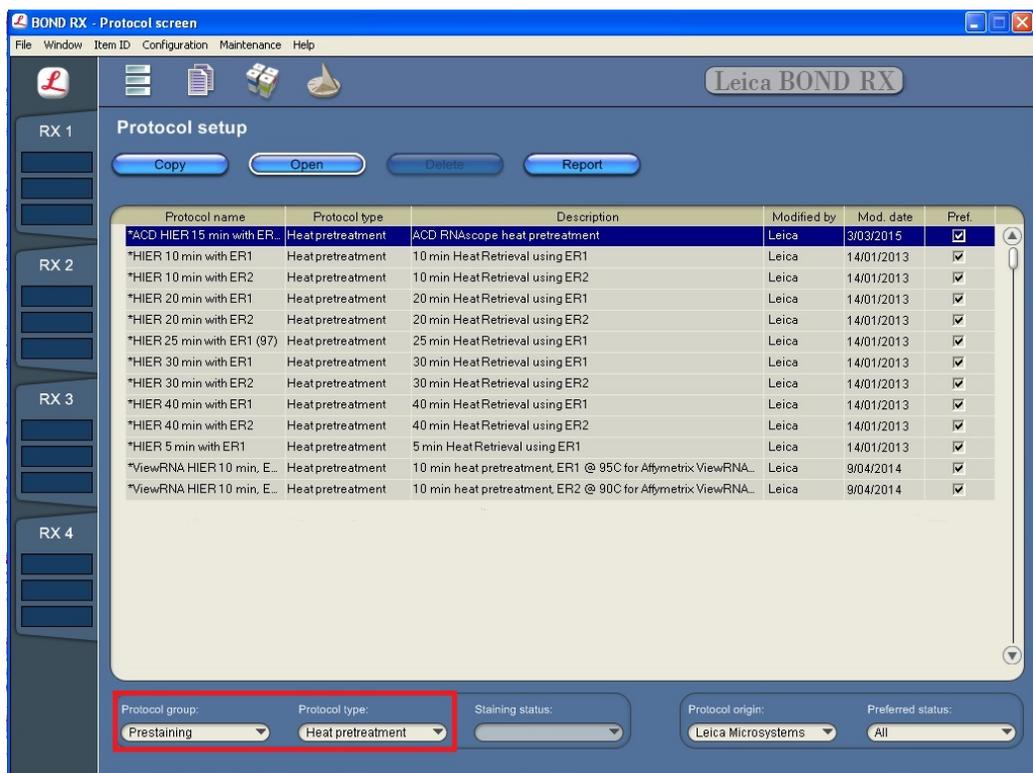
The following example shows how to edit the Epitope Retrieval procedure from within the software.

### Create a prestaining protocol

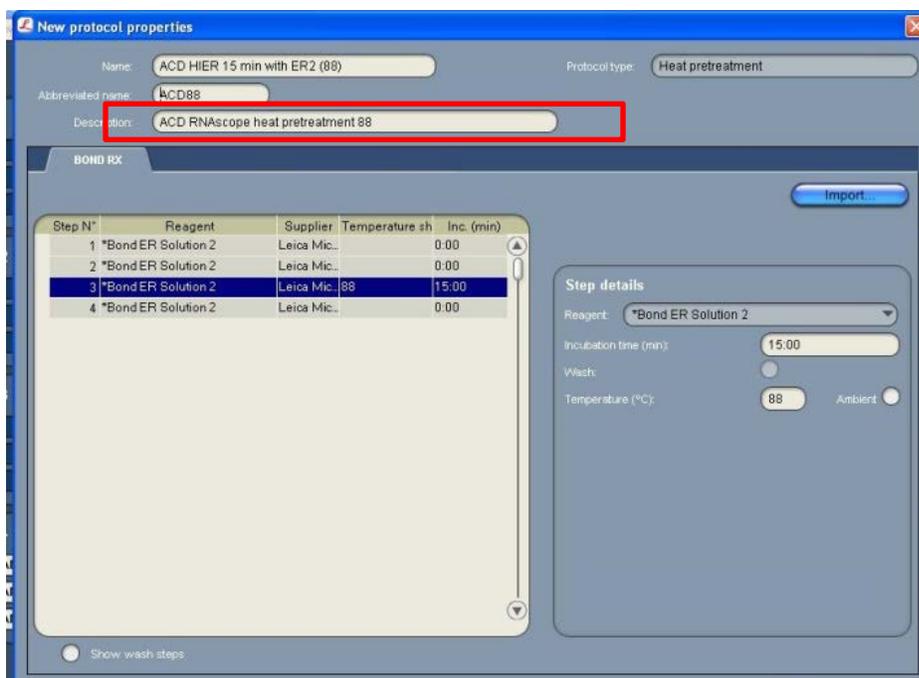
1. Open the Leica BOND software and click on the **Protocol setup** icon as shown.



2. Select **Prestaining** under the Protocol group menu and **Heat pretreatment** under the Protocol type menu to access the heat pretreatment protocols.



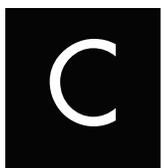
3. Highlight the **\*ACD HIER 15 min with ER2 (95)** protocol. Select **Copy**.
- Note:** ER2 = Epitope Retrieval 2.
4. Rename the protocol as **ACD HIER 15 min with ER2 (88)**.
  5. Rename the Abbreviated name as **ER2-88**.
  6. Rename the Description to **ACD RNAScope heat pretreatment 88**.



7. Highlight the third **\*Bond ER Solution 2** step (see above). Depending on the tissue type used, change the temperature and time as shown in the following table.

Tissue Type	ER2 Incubation Time	Temperature
Brain and spinal cord	15 MIN	95°C
Breast cancer	15 MIN	95°C
Cell pellet	15 MIN	88°C
Colon	15 MIN	95°C
GI tract	15 MIN	95°C
Head and neck cancer	15 MIN	95°C
Heart	15 MIN	95°C
Kidney	15 MIN	95°C
Liver	15 MIN	95°C
Lung	15 MIN	95°C
Lymphoma	15 MIN	95°C
Placenta	15 MIN	95°C
Prostate	15 MIN	95°C
Skin	15 MIN	95°C
Stomach	15 MIN	95°C
Thymus	15 MIN	88°C or 95°C
Tonsil	15 MIN	88°C or 95°C
Xenograft	15 MIN	88°C
Mouse tissue	15 MIN	88°C or 95°C

8. Select **Save** to create a protocol for ER2 pretreatment at 88°C.  
9. If needed, repeat Steps 1 through 8 to create a new heating protocol (for example, ACD 25min ER2).



# Appendix C. Edit the Protease Protocol

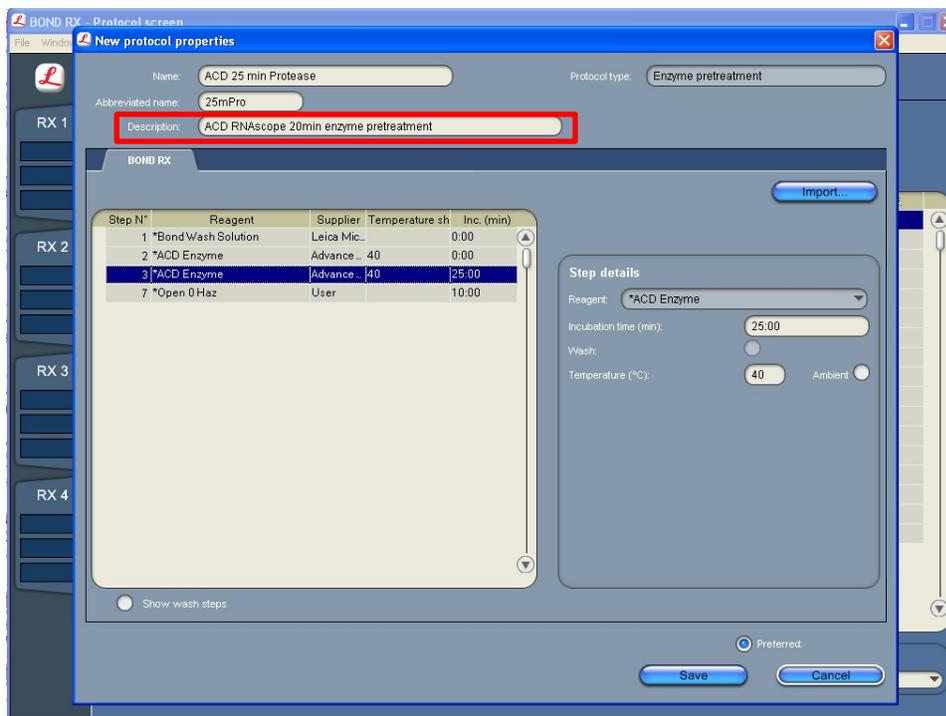
The following example shows how to edit the Protease procedure from within the software.

1. Select **Enzyme Pretreatment** under the Protocol type menu (bottom left).
2. Highlight the **\*ACD 15min Protease** protocol. Select **Copy**.

Protocol name	Protocol type	Description	Modified by	Mod. date	Pref.
*ACD 15min Protease	Enzyme pretreatment	ACD RNAscope enzyme pretreatment	Leica	3/03/2015	<input checked="" type="checkbox"/>
*Enzyme 1 (20)	Enzyme pretreatment	Enzyme 1 pretreatment (20 min) for ViewRNA eZ-L Assay	Leica	3/03/2015	<input type="checkbox"/>
*Enzyme 1 for 10 min	Enzyme pretreatment	10 min Enzyme Pretreatment using Enzyme 1	Leica	14/01/2013	<input checked="" type="checkbox"/>
*Enzyme 1 for 15 min	Enzyme pretreatment	15 min Enzyme Pretreatment using Enzyme 1	Leica	14/01/2013	<input checked="" type="checkbox"/>
*Enzyme 1 for 5 min	Enzyme pretreatment	5 min Enzyme Pretreatment using Enzyme 1	Leica	14/01/2013	<input checked="" type="checkbox"/>
*Enzyme 2 (20)	Enzyme pretreatment	Enzyme 2 pretreatment (20 min) for ViewRNA eZ-L Assay	Leica	3/03/2015	<input type="checkbox"/>
*Enzyme 2 for 10 min	Enzyme pretreatment	10 min Enzyme Pretreatment using Enzyme 2	Leica	14/01/2013	<input checked="" type="checkbox"/>
*Enzyme 2 for 15 min	Enzyme pretreatment	15 min Enzyme Pretreatment using Enzyme 2	Leica	14/01/2013	<input checked="" type="checkbox"/>
*Enzyme 3 (20)	Enzyme pretreatment	Enzyme 3 pretreatment (20 min) for ViewRNA eZ-L Assay	Leica	3/03/2015	<input type="checkbox"/>
*Enzyme 3 for 10 min	Enzyme pretreatment	10 min Enzyme Pretreatment using Enzyme 3	Leica	14/01/2013	<input checked="" type="checkbox"/>
*Enzyme 3 for 15 min	Enzyme pretreatment	15 min Enzyme Pretreatment using Enzyme 3	Leica	14/01/2013	<input checked="" type="checkbox"/>
*Enzyme 5 for 25 min	Enzyme pretreatment	25 min Enzyme Pretreatment using Enzyme 5	Leica	14/01/2013	<input checked="" type="checkbox"/>
*Protease 20 min and fix	Enzyme pretreatment	Protease 20 min and fix	Leica	9/04/2014	<input checked="" type="checkbox"/>
*ViewRNA Enzyme 1 (20)	Enzyme pretreatment	20 min enzyme pretreatment for Affymetrix ViewRNA eZ Assay	Leica	9/04/2014	<input checked="" type="checkbox"/>
*ViewRNA Enzyme 2 (20)	Enzyme pretreatment	20 min enzyme pretreatment for Affymetrix ViewRNA eZ Assay	Leica	9/04/2014	<input checked="" type="checkbox"/>
*ViewRNA Enzyme 3 (20)	Enzyme pretreatment	20 min enzyme pretreatment for Affymetrix ViewRNA eZ Assay	Leica	9/04/2014	<input checked="" type="checkbox"/>

Protocol group: Prestaining | Protocol type: **Enzyme pretreatment** | Staining status: | Protocol origin: Leica Microsystems | Preferred status: All

3. Rename the protocol to **ACD 25min Protease**.
4. Rename the Abbreviated name to **25mPro**.
5. Rename the Description to **ACD RNAscope 25min enzyme pretreatment**.



- Highlight the second **\*ACD Enzyme** step. Keep the temperature at **40°C** and set the enzyme incubation time to **15 MIN** for the following:

Tissue Type
Brain and spinal cord
Breast cancer
Cell pellet
Colon
GI tract
Head and neck cancer
Heart
Kidney
Liver
Lung
Lymphoma
Placenta
Prostate
Skin
Stomach
Thymus
Tonsil
Xenograft

- Select **Save**.
- If needed, repeat Steps 1 through 7 to create a new protease protocol for different sample types (for example, ACD 10min Protease or ACD 15min Protease at ambient temperature).



# Appendix D. Safety

## Chemical safety



**WARNING!** GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below, and consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see <https://acdbio.com/technical-support/user-manuals>.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

## Biological hazard safety



**WARNING!** BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the following:

### In the U.S.:

- U.S. Department of Health and Human Services guidelines published in Biosafety in Microbiological and Biomedical Laboratories found at: <https://www.cdc.gov/biosafety/>
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR § 1910.1030), found at: [https://www.osha.gov/pls/oshaweb/owadisp.show\\_document?p\\_id=10051&p\\_table=STANDARDS](https://www.osha.gov/pls/oshaweb/owadisp.show_document?p_id=10051&p_table=STANDARDS)

- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.
- Additional information about biohazard guidelines is available at:  
**<https://www.cdc.gov/biosafety/>**

#### **In the EU:**

- Check local guidelines and legislation on biohazard and biosafety precaution and refer to the best practices published in the World Health Organization (WHO) Laboratory Biosafety Manual, third edition, found at:  
**[http://www.who.int/csr/resources/publications/biosafety/WHO\\_CDS\\_CSR\\_LYO\\_2004\\_11/en/](http://www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/)**
- Information about the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) can be found at: **<https://echa.europa.eu/regulations/reach>**

# Documentation and Support

## Obtaining SDSs

Safety Data Sheets (SDSs) are available at: <https://acdbio.com/technical-support/user-manuals>. For the SDSs of chemicals not distributed by Advanced Cell Diagnostics, contact the chemical manufacturer.

## Obtaining support

For the latest services and support information, go to: <https://acdbio.com/technical-support/support-overview>.

At the website, you can:

- Access telephone and fax numbers to contact Technical Support and Sales facilities.
- Search through frequently asked questions (FAQs).
- Submit a question directly to Technical Support.
- Search for user documents, SDSs, application notes, citations, training videos, and other product support documents.
- Find out information about customer training events.

## Contact information

Advanced Cell Diagnostics, Inc.

7707 Gateway Blvd Suite 200

Newark, CA 94545

Toll Free: 1-877-576-3636

Direct: 1-510-576-8800

Fax: 1-510-576-8801

Information: [info.acd@bio-techne.com](mailto:info.acd@bio-techne.com)

Orders: [orders.acd@bio-techne.com](mailto:orders.acd@bio-techne.com)

Support Email: [support.acd@bio-techne.com](mailto:support.acd@bio-techne.com)

## Limited product warranty

Advanced Cell Diagnostics, Inc. and/or its affiliate(s) warrant their products as set forth in the ACD General Terms and Conditions of Sale found on the ACD website. If you have any questions, please contact Advanced Cell Diagnostics at <https://acdbio.com/about/contact>.

**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-technie.com](mailto:support.acd@bio-technie.com)

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

