



# miRNAscope™ LS Reagent Kit - RED

For use with Leica Biosystems' BOND RX System

Document Number UM 324600

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#### **Citing RNAscope<sup>®</sup> in Publications**

When describing a procedure for publication using this product, please refer to it as the RNAscope<sup>®</sup> Assay and cite: Wang F, Flanagan J, Su N, Wang L-C, Bui S, Nielson A, Wu X, Vo H-T, Ma X-J and Luo Y. RNAscope<sup>®</sup>: A Novel *In Situ* RNA Analysis Platform for Formalin-Fixed Paraffin-Embedded Tissues. *J. Mol. Diagnostics*, 2012, 14:22–29.

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# Contents

<b>Chapter 1. Product Information</b> .....	<b>5</b>
About this guide.....	5
Product description.....	5
Background.....	5
Overview.....	5
Kit contents and storage.....	6
miRNAscope™ LS Probes.....	6
miRNAscope™ LS Reagents.....	7
Required materials and equipment from Leica BOND RX.....	7
User-supplied materials.....	8
<b>Chapter 2. Before You Begin</b> .....	<b>9</b>
Important procedural guidelines.....	9
<b>Chapter 3. Prepare and Pretreat Samples</b> .....	<b>10</b>
Prepare FFPE sections.....	10
Materials required.....	10
Fix the sample.....	10
Dehydrate, embed, and cut the sample.....	10
Prepare fresh-frozen sections.....	11
Materials required.....	11
Fix the sections.....	11
Dehydrate the sections.....	11
<b>Chapter 4. Set Up Software Version 5.2</b> .....	<b>12</b>
Workflow.....	12
Register the reagents.....	13
Create a one-minute probe hybridization protocol.....	14
Create a staining protocol.....	16
Register the mock probe.....	21
Set up a study.....	23
<b>Chapter 5. Set Up Software Version 6.0</b> .....	<b>27</b>
Workflow.....	27
Register the reagents.....	28
Create the miRNA probe hybridization protocol.....	30
Register the probes.....	31
Create the staining protocol.....	33
Set up a study.....	37

<b>Chapter 6. Run the miRNAscope™ LS Assay</b> .....	<b>41</b>
Workflow .....	41
Materials required.....	42
Prepare the instrument .....	42
Prepare the instrument reagents.....	42
Start the run .....	43
Complete the run.....	43
Mount the samples .....	44
<b>Chapter 7. Evaluate the Results</b> .....	<b>45</b>
Evaluate the samples .....	45
Scoring guidelines.....	45
Staining examples.....	46
Troubleshooting.....	46
<b>Appendix A. Edit the Epitope Retrieval Protocol</b> .....	<b>47</b>
Create a prestaining protocol.....	47
<b>Appendix B. Edit the Protease Protocol</b> .....	<b>50</b>
<b>Appendix C. Safety</b> .....	<b>52</b>
Chemical safety.....	52
Biological hazard safety .....	52
<b>Documentation and Support</b> .....	<b>54</b>
Obtaining SDSs .....	54
Obtaining support.....	54
Contact information.....	54
Limited product warranty .....	54

# 1

## Chapter 1. Product Information



Before using this product, read and understand the information in **Appendix E. Safety** on page 52 of 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 miRNAscope™ LS Reagent Kit – RED (Cat. No. 324600) for use with Leica Biosystems' BOND RX Research Advanced Staining System. miRNAscope™ LS Assays are compatible with a variety of sample types.

### Product description

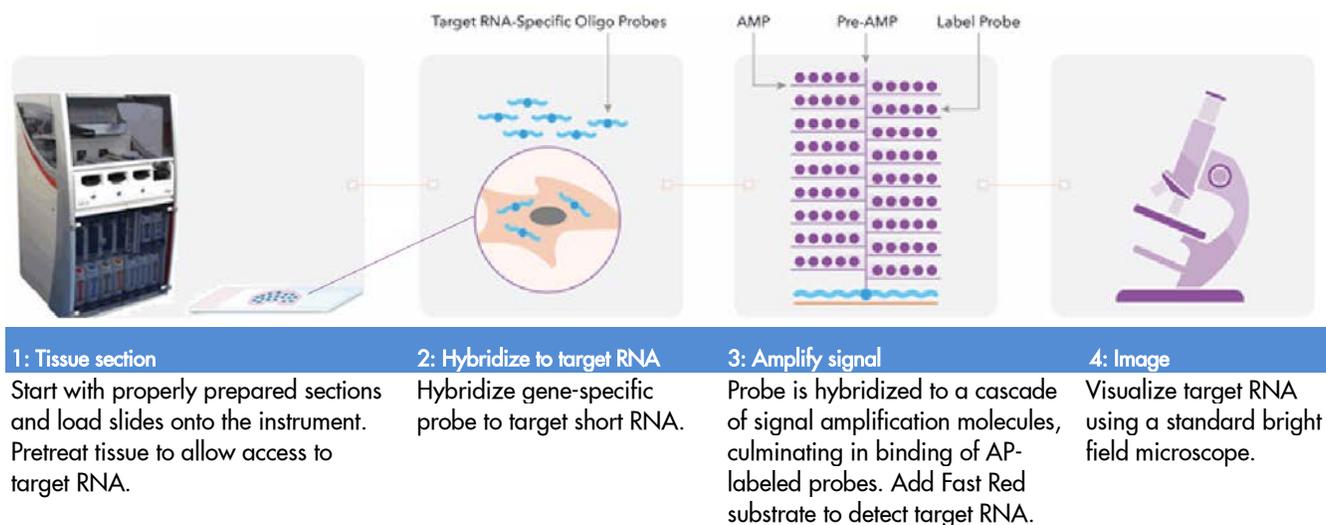
#### Background

The miRNAscope™ LS Assays use a novel and proprietary method of in situ hybridization (ISH) to visualize microRNAs (miRNAs), PIWI-interacting RNAs (piRNAs), short interfering RNAs (siRNAs), and antisense oligonucleotides (ASOs) with single cell resolution in samples mounted on slides. The assays are based on ACD's patented signal amplification and background suppression technology and can detect RNA molecules (17-50 nucleotides) in archival samples and partially degraded specimens. The miRNAscope™ LS Assay allows users to automate the highly sensitive miRNAscope Assay using Leica Biosystems' BOND RX System.

#### Overview

See **Figure 1** on page 6 for an illustration of the assay, which can be completed on the instrument in ~9–10 hours. Starting with properly prepared samples, sections are first pretreated, and then RNA-specific probes are hybridized to target RNA. The signal is amplified using a multi-step process, followed by hybridization to alkaline phosphatase (AP)-labeled probes and detected using the Fast Red chromogenic substrate. The miRNAscope™ signal appears as a distinct dot of chromogen precipitate visible using a common bright-field microscope.

**Figure 1.** Procedure overview



## Kit contents and storage

The miRNAscope™ LS Assay requires the miRNAscope™ LS Probes and the miRNAscope™ LS Reagents, available from Advanced Cell Diagnostics. Probes and reagent kits are available separately.

### miRNAscope™ LS Probes

The miRNAscope™ 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. Visit <http://www.acdbio.com/control-slides-and-probes> to order appropriate control probes. Each probe is sufficient for staining ~30 standard slides. The probes have a shelf life of two years from the manufacturing date when stored as indicated in the following tables:

Target Probes					
<input checked="" type="checkbox"/>	Reagent	Cat. No.	Content	Quantity	Storage
	miRNAscope™ 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
	miRNAscope™ LS Positive Control Probe - SR-RNU6-S1	727878-S1	Probe targeting common housekeeping gene	16 mL x 1 bottle	2–8°C
	miRNAscope™ LS Negative Control Probe - SR-Scramble-S1	727888-S1	Scramble probe	16 mL x 1 bottle	2–8°C

## miRNAscope™ LS Reagents

The miRNAscope™ LS Reagent Kit (Cat. No. 324600) contains all the reagents needed to run the miRNAscope™ LS Assay on 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 tables:

miRNAscope™ LS Reagent Kit (Cat. No. 324600)			
<input checked="" type="checkbox"/>	Reagent	Quantity	Storage
	RNAAscope® 2.5 LS Hydrogen Peroxide	21 mL x 1 bottle	2–8°C
	RNAAscope® 2.5 LS Protease III	21 mL x 1 bottle	2–8°C
	RNAAscope® 2.5 LS Rinse	29 mL x 2 bottles	2–8°C
	RNAAscope® 2.5 LS Bluing Reagent	21 mL x 1 bottle	2–8°C
	miRNAscope™ LS Amp 1	21 mL x 1 bottle	2–8°C
	miRNAscope™ LS Amp 2	21 mL x 1 bottle	2–8°C
	miRNAscope™ LS Amp 3	21 mL x 1 bottle	2–8°C
	miRNAscope™ LS Amp 4–Red	21 mL x 1 bottle	2–8°C
	miRNAscope™ LS Amp 5–Red	21 mL x 1 bottle	2–8°C
	miRNAscope™ LS Amp 6–Red	21 mL x 1 bottle	2–8°C

**IMPORTANT!** Use only miRNAscope™ LS Probes. Do not substitute the reagent components of the miRNAscope™ LS Reagent Kit with those of other reagent kits. Required materials and equipment

**Note:** Bluing is an optional step.

## Required materials and equipment from Leica BOND RX

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

<input checked="" type="checkbox"/>	Component	Cat. No.	Storage
	Leica Biosystems' BOND RX System — automated slide stainer	—	—
	BOND Open containers 30 mL	OP309700	Room temp (20–25°C)
	BOND Universal Covertiles 100 pack	S21.2001	Room temp (20–25°C)
	BOND Mixing Stations	S21.1971	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 Aspirating Probe Cleaning System	CS9100	2–8°C
	BOND Polymer Refine Red Detection and Hematoxylin*	DS9390	2–8°C

\* Do not substitute with any other chromogen kit.

## 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
	100% alcohol (EtOH)	American Master Tech Scientific/MLS*	ALREACS
	Xylene	Fisher Scientific/MLS	X3P-1GAL
	10% Neutral Buffered Formalin (NBF)/ 4% Paraformaldehyde (PFA)	MLS	—
	Paraffin wax	MLS	—
	1X Phosphate Buffered Saline (PBS)	MLS	—
	Microtome	MLS	—
	Drying oven, capable of holding temperature at 60 +/- 1°C (optional)	MLS	—
	Ammonium hydroxide, 28–30% (optional)	Sigma-Aldrich/MLS	320145 500mL
	EcoMount	Biocare Medical	EM897L
	Tissue-Tek® Vertical 24 Slide Rack	American Master Tech Scientific/MLS	LWSRA24
	Tissue-Tek® Staining Dishes	American Master Tech Scientific/MLS	LWT4457EA
	Tissue-Tek® Clearing Agent Dishes, xylene resistant	American Master Tech Scientific/MLS	LWT4456EA
	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 miRNAscope™ 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 FFPE Control Slides (Cat. No. 310045 for Human HeLa Cell Pellet, and Cat. No. 310023 for Mouse 3T3 Cell Pellet) using the miRNAscope™ LS positive and negative control probes.

### Important procedural guidelines

- Start with properly fixed and prepared sections. Refer to **Chapter 3. Prepare and Pretreat Samples** on page 10 for preparation of FFPE slides. For preparation of other sample types, contact [support.acd@bio-technne.com](mailto:support.acd@bio-technne.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 E. Safety** on page 52 for more information.

# 3

## Chapter 3. Prepare and Pretreat Samples

The following protocols describe formalin-fixed, paraffin-embedded (FFPE) and fresh-frozen sample preparation. For other sample types and preparation methods, contact [support.acd@bio-technne.com](mailto:support.acd@bio-technne.com) for the latest protocols and guidelines.

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

### Prepare FFPE sections

#### Materials required

- 10% Neutral Buffered Formalin (NBF)
- 1X PBS
- Paraffin wax
- Tissue-Tek® Clearing Agent Dishes
- Tissue-Tek® Staining Dishes
- Tissue-Tek® Vertical 24 Slide Rack
- 100% alcohol (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 10% NBF within **1 HR** of biopsy.
3. Fix the tissue for **16–32 HRS** at **ROOM TEMPERATURE (RT)**. Fixation time will vary depending on tissue type and size.



**CAUTION!** Handle biological specimens appropriately.

**IMPORTANT!** Fixation for **<16 HRS** or **>32 HRS** will impair the performance of the assay.

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

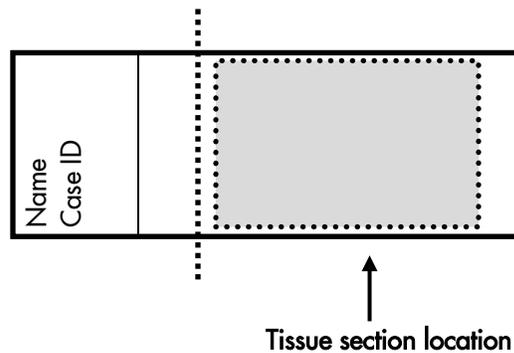
**IMPORTANT!** Use fresh reagents.

4. Wash sample with 1X PBS.
5. Dehydrate sample using a standard ethanol series, followed by xylene.
6. Embed sample in paraffin using standard procedures.

**Note:** Store embedded samples at room temperature with desiccation. To better preserve RNA quality over a long period (>1 yr), we recommend storing at 2–8°C with desiccation.

7. Trim paraffin blocks as needed and cut embedded tissue into 5 +/- 1 µm sections using a microtome.

8. 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:




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

---

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

---

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

---

## Prepare fresh-frozen sections

### Materials required

- 1X PBS
- 10% Neutral Buffered Formalin (NBF) or 4% Paraformaldehyde (PFA)
- 100% alcohol (EtOH)
- Tissue-Tek® Vertical 24 Slide Rack
- Tissue-Tek® Staining Dishes

### Fix the sections

1. Remove fresh-frozen tissue slides from **–80°C** and place in a Tissue Tek® Slide Rack.
2. *Immediately* immerse the slides in 200 mL of 10% NBF or 4% PFA.
3. Incubate the slides for at least **90 MIN** at **ROOM TEMPERATURE (RT)**.

**Note:** Formalin that has been stored for more than six months, exposed to air for more than a week, or used repeatedly may result in suboptimal tissue fixation.

### Dehydrate the sections

Reagents may be prepared ahead of time. Ensure all containers remain covered.

1. Prepare 200 mL 50% ethanol, 200 mL 70% ethanol, and 2X 200 mL 100% ethanol in Tissue Tek® Staining Dishes.
2. Place the slides in 50% ethanol for **5 MIN** at **RT**.
3. Place the slides in 70% ethanol for **5 MIN** at **RT**.
4. Place the slides in 100% ethanol for **5 MIN** at **RT**.
5. Place slides in fresh 100% ethanol for **5 MIN** at **RT**.
6. Remove slides from ethanol, and let them dry for **5 MIN** at **RT**.

# 4

## Chapter 4. Set Up Software Version 5.2

Use the instructions in this chapter to set up the miRNAscope™ LS Assay using software version 5.2. To set up the assay using software version 6.0, proceed to **Chapter 5** on page 27.

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**IMPORTANT!** BXD11 or higher is required to run the following setup on software version 5.2. Please contact your Leica FAS to upgrade to BXD11 before starting the assay.

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

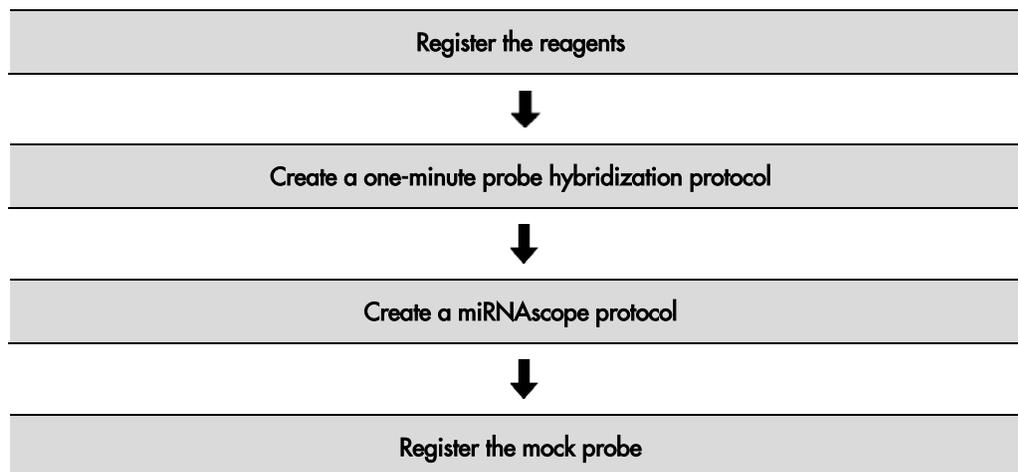
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**IMPORTANT!** Make sure you have software version 5.2 or 6.0 before setting up the assay. The miRNAscope™ LS Assay is not supported by software version 4.0.

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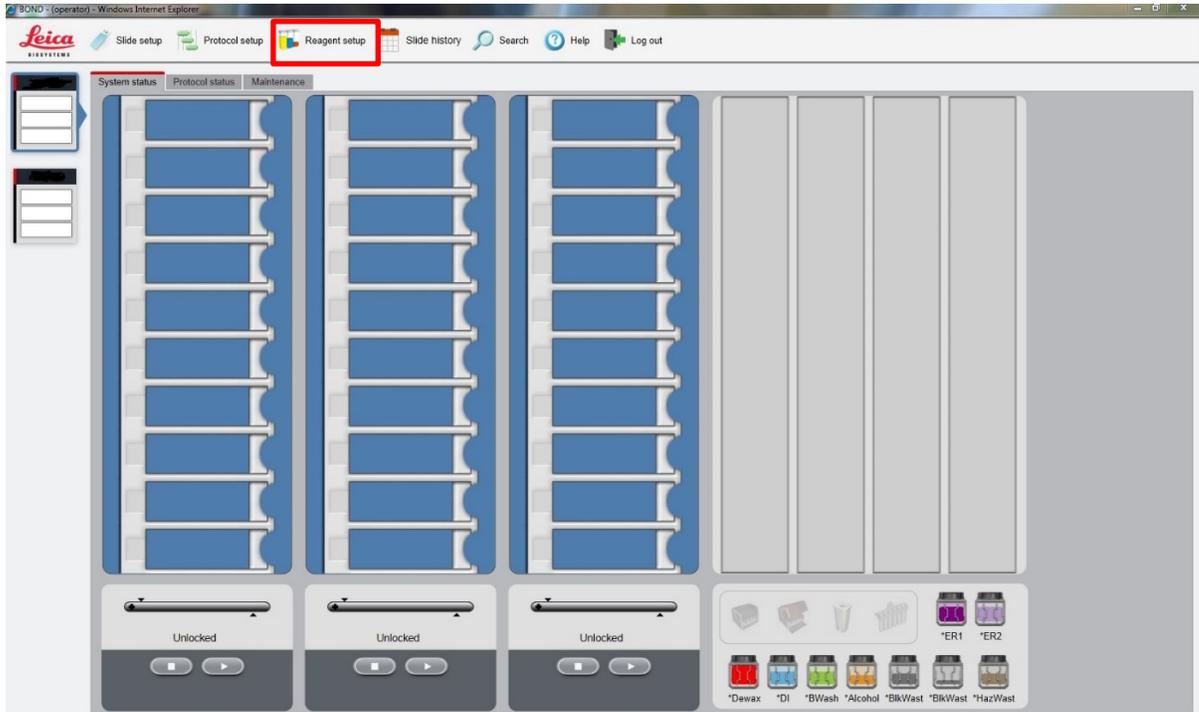
### Workflow



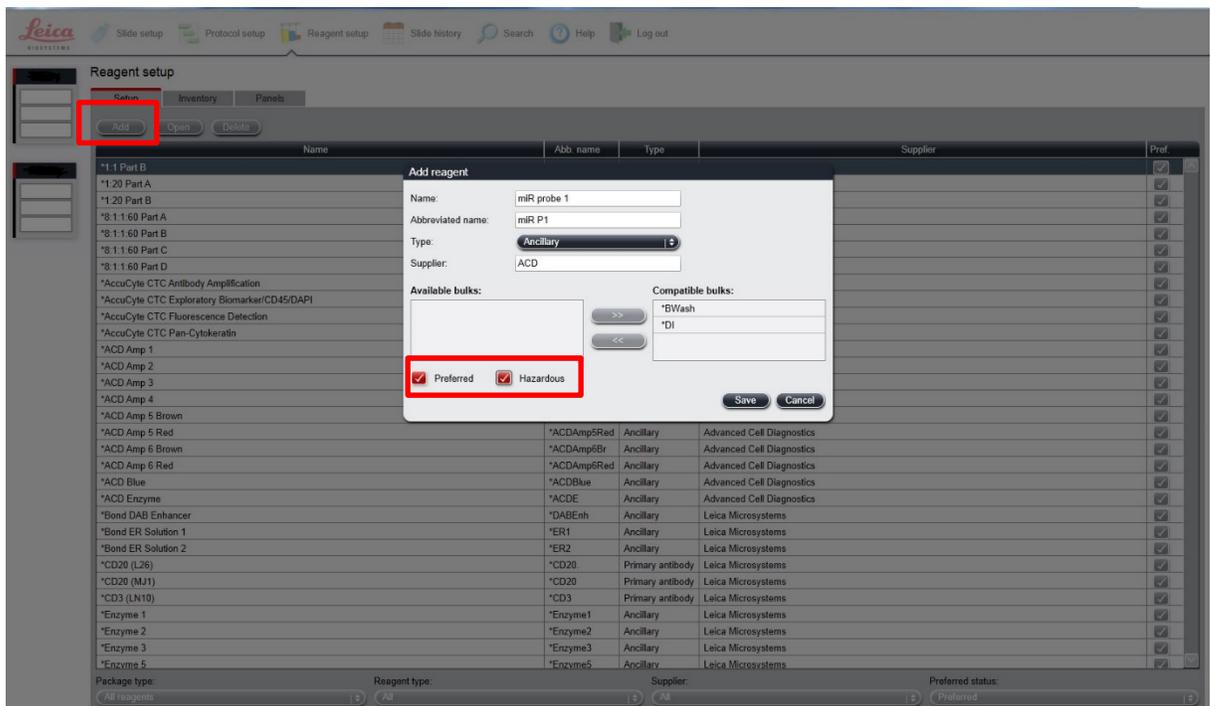
## Register the reagents

This step is a “workaround” to the existing 5.2 software to accommodate the miRNAscope™ LS Assay. Your ACD Field Application Specialist (FAS) should implement this procedure. In summary, a probe is created as an ancillary reagent and 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. Enter a reagent name (for example, **miR probe 1**) in the Name text box.

4. Enter **miR P1** (for example) in the Abbreviated name text box.
5. Select **Ancillary** in the Type drop-down menu.
6. Enter **ACD** in the Supplier text box.
7. Check both the **Preferred** and **Hazardous** boxes (for the probes, miRNAscope™ Amp 1, and miRNAscope™ Amp 3 only).
8. Select **Save**.
9. Repeat Steps 2–7 for each probe and for the rest of the reagents using the container names in the following table:

Reagent	Container Name	Abbreviated Name
miRNAscope™ LS Amp 1	miRNAscope Amp 1	miR Amp 1
miRNAscope™ LS Amp 2	miRNAscope Amp 2	miR Amp 2
miRNAscope™ LS Amp 3	miRNAscope Amp 3	miR Amp 3
miRNAscope™ LS Amp 4–Red	miRNAscope Amp 4 Red	miR Amp 4
miRNAscope™ LS Amp 5–Red	miRNAscope Amp 5 Red	miR Amp 5
miRNAscope™ LS Amp 6–Red	miRNAscope Amp 6 Red	miR Amp 6

**Note:** Complete these steps only once for each reagent, including probes. Bluing is optional and can be completed off the instrument.

## Create a one-minute probe hybridization protocol

A mock probe hybridization step must be created as part of the version 5.2 software workaround for the miRNAscope™ LS Assay. The following example copies the existing two hour hybridization protocol and changes the incubation time to one minute and the temperature to ambient.

**Note:** If an existing one-minute probe hybridization protocol already exists, skip to the next section.

1. In the Protocol setup screen, select **Prestaining** from the Protocol group menu and **ISH Hybridization** from the Protocol type menu.
2. Highlight the **\*ACD 2 hour Hybridization** protocol. Select **Copy**.

**Protocol setup**

Copy Open Delete Report

Protocol name	Protocol type	Description	Modified by	Mod. date	Pref.
*ACD 2 hour Hybridization	ISH hybridization	ACD RNAscope 2 hour hybridization	Leica	3/21/2018	<input checked="" type="checkbox"/>
*ISH Hybridization (12H)	ISH hybridization	ISH Hybridization protocol for 12 hours	Leica	3/21/2018	<input checked="" type="checkbox"/>
*ISH Hybridization (2H)	ISH hybridization	ISH Hybridization protocol	Leica	3/21/2018	<input checked="" type="checkbox"/>
*RNAscope 2.5 LSx Hybridization	ISH hybridization	RNAscope 2.5 LSx 2 hour Hybridization	Leica	3/21/2018	<input checked="" type="checkbox"/>
*ViewRNA Probe Hybridization (3Hr)	ISH hybridization	3 hour probe hybridization for Affymetrix ViewRNA eZ Assay	Leica	3/21/2018	<input checked="" type="checkbox"/>

Protocol group: Prestaining Protocol type: ISH hybridization Staining status: Protocol origin: Leica Microsystems Preferred status: Preferred

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

**New protocol properties**

Name: ACD 1min Hybridization  
Abbreviated name: ACD1min  
Description: ACD 1min RNAscope hybridization Import...

BOND RX<sup>m</sup> BOND RX Protocol type: ISH hybridization

Step N°	Reagent	Supplier	Temperature	Inc. (min)
1	*No Reagent	Leica Microsystems	Ambient	1:00

**Step details**

Reagent: \*No Reagent  
Incubation time (min): 1:00  
 Wash  
Temperature (°C):  
 Ambient  
Dispense type: Intermediate

Show wash steps

Preferred Save Cancel

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

## Create a staining protocol

Due to the 5.2 software workaround for the miRNAscope™ LS Assay, unique staining protocols *must be created for each probe*.

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

The screenshot shows the 'Protocol setup' window with a table of protocols. The 'ACD 2.5 Red Rev B' protocol is highlighted. The 'Copy' button is circled in red. At the bottom, the 'Protocol group' dropdown menu is also circled in red and set to 'Staining'.

Protocol name	Protocol type	Description	Modified by	Mod. date	Prof.
*IF Protocol	IHC staining	IF protocol	Leica	3/21/2018	✓
*IHC Open Dispense Template	IHC staining	IHC template with Open Ancillary and Chromogen dispenses	Leica	3/21/2018	✓
*IHC Protocol F	IHC staining	Bond Polymer Refine IHC protocol	Leica	3/21/2018	✓
*IHC Protocol F RX 37M	IHC staining	IHC Protocol F with marker step at 37C	Leica	3/21/2018	✓
*IHC Protocol F RX 40M	IHC staining	IHC Protocol F with marker step at 40C	Leica	3/21/2018	✓
*IHC Protocol J	IHC staining	Bond Polymer Refine Red IHC protocol	Leica	3/21/2018	✓
*IHC Protocol J RX 37M	IHC staining	IHC Protocol J with marker step at 37C	Leica	3/21/2018	✓
*IHC Protocol J RX 40M	IHC staining	IHC Protocol J with marker step at 40C	Leica	3/21/2018	✓
*IHC Protocol K	IHC staining	ChromoPlex 1 Dual IHC protocol	Leica	3/21/2018	✓
*IHC Protocol K - 50 Test	IHC staining	ChromoPlex 1 Dual IHC protocol	Leica	3/21/2018	✓
*ACD 2.5 DAB Rev B	ISH detection	ACD 2.5 DAB Rev B protocol	Leica	3/21/2018	✓
<b>*ACD 2.5 Red Rev B</b>	<b>ISH detection</b>	<b>ACD 2.5 Red Rev B protocol</b>	Leica	3/21/2018	✓
*ACD DAB ISH Protocol	ISH detection	ACD RNAscope DAB ISH protocol	Leica	3/21/2018	✓
*ACD Red ISH Protocol	ISH detection	ACD RNAscope Red ISH protocol	Leica	3/21/2018	✓
*FISH A RUO Protocol	ISH detection	Bond RUO FISH A protocol	Leica	3/21/2018	✓
*FISH Protocol C	ISH detection	FISH wash protocol	Leica	3/21/2018	✓
*Flexible ISH Template	ISH detection	ISH template with open dispenses plus heated wash steps	Leica	3/21/2018	✓
*ISH Heated Wash Template	ISH detection	ISH template with multiple wash temperatures	Leica	3/21/2018	✓
*ISH Open Dispense Template	ISH detection	ISH template with Open Ancillary and Chromogen dispenses	Leica	3/21/2018	✓
*ISH Protocol A	ISH detection	Bond Polymer Refine RNA ISH protocol	Leica	3/21/2018	✓
*ISH Protocol B	ISH detection	Bond Polymer Refine DNA ISH protocol	Leica	3/21/2018	✓
*RNAscope 2.5 LSx DAB ISH	ISH detection	RNAscope 2.5 LSx DAB ISH protocol	Leica	3/21/2018	✓
*RNAscope 2.5 LSx RED ISH	ISH detection	RNAscope 2.5 LSx RED ISH protocol	Leica	3/21/2018	✓
*TUNEL Template	ISH detection	TUNEL assay template	Leica	3/21/2018	✓
*ViewRNA 1 - FFPE	ISH detection	Affymetrix ViewRNA eZ Assay, FFPE Protocol 1	Leica	3/21/2018	✓
*ViewRNA 2	ISH detection	Affymetrix ViewRNA eZ Assay, Alternate 2	Leica	3/21/2018	✓
*ViewRNA 3	ISH detection	Affymetrix ViewRNA eZ Assay, Alternate 3	Leica	3/21/2018	✓

Protocol group: Staining | Protocol type: All | Staining status: All | Protocol origin: Leica Microsystems | Preferred status: Preferred

3. Change the protocol name for your first probe to **ACD miR ISH Red P1** in the Name text box, **RedmiRP1** in the Abbreviated name text box, and **ACD miRNAscope ISH Red Probe 1** in the Description text box.

- To edit the staining protocol, make sure to select the correct tab that matches your instrument type (BOND RX<sup>m</sup> or BOND RX).

New protocol properties

Name: ACD miR ISH Red P1  
 Abbreviated name: RedmiRP1  
 Description: ACD miRNAscope ISH Red Probe 1 Import...

BOND RX<sup>m</sup> **BOND RX** Protocol type: ISH detection

Preferred detection system: Bond Polymer Refine Red Detection

Step N°	Reagent	Supplier	Temperature	Inc. (min)
1	miR probe 1	ACD	Ambient	0:00
2	miR probe 1	ACD	Ambient	0:00
3	miR probe 1	ACD	37	120:00
15	miRNAscope Amp 1	ACD	42	1:00
16	miRNAscope Amp 1	ACD	42	30:00
25	*LS Rinse	Advanced Cell Diagnostics	Ambient	5:00
26	*LS Rinse	Advanced Cell Diagnostics	Ambient	5:00
31	miRNAscope Amp 2	ACD	42	1:00
32	miRNAscope Amp 2	ACD	42	15:00
41	miRNAscope Amp 3	ACD	42	1:00
42	miRNAscope Amp 3	ACD	42	30:00
51	*LS Rinse	Advanced Cell Diagnostics	Ambient	5:00

Step details

Reagent: miRNAscope Amp 3 (ACD)  
 Incubation time (min): 30:00  
 Wash  
 Temperature (°C): 42  
 Ambient  
 Dispense type: 150 µL

Insert wash Insert reagent Delete step  Show wash steps

Staining method:  Single  First  Second  Preferred

Save Cancel

New protocol properties

Name: ACD miR ISH Red P1  
 Abbreviated name: RedmiRP1  
 Description: ACD miRNAscope ISH Red Probe 1 Import...

BOND RX<sup>m</sup> **BOND RX** Protocol type: ISH detection

Preferred detection system: Bond Polymer Refine Red Detection

Step N°	Reagent	Supplier	Temperature	Inc. (min)
52	*LS Rinse	Advanced Cell Diagnostics	Ambient	5:00
57	miRNAscope Amp 4 Red	ACD	42	1:00
58	miRNAscope Amp 4 Red	ACD	42	15:00
67	miRNAscope Amp 5 Red	ACD	Ambient	1:00
68	miRNAscope Amp 5 Red	ACD	Ambient	15:00
77	miRNAscope Amp 6 Red	ACD	Ambient	1:00
78	miRNAscope Amp 6 Red	ACD	Ambient	15:00
87	*LS Rinse	Advanced Cell Diagnostics	Ambient	5:00
88	*LS Rinse	Advanced Cell Diagnostics	Ambient	5:00
89	*Mixed Red Refine	Leica Microsystems	Ambient	1:00
90	*Mixed Red Refine	Leica Microsystems	Ambient	10:00
98	*Hematoxylin	Leica Microsystems	Ambient	5:00

Step details

Reagent: miRNAscope Amp 6 Red (ACD)  
 Incubation time (min): 15:00  
 Wash  
 Temperature (°C):  
 Ambient  
 Dispense type: 150 µL

Insert wash Insert reagent Delete step  Show wash steps

Staining method:  Single  First  Second  Preferred

Save Cancel

**Note:** The preceding two figures display all reagent steps.

5. Click on **Show wash steps** to also view the wash steps.
6. To edit each step, highlight and select it. Set up each step, including wash steps, according to the following table.

**IMPORTANT!** To assure the success of the assay, change the temperature of Step 3 to **37°C**.

Step No.	Reagent	Step Type	Incubation Time	Temperature
1	miR Probe 1	Reagent	0 MIN	Ambient
2	miR Probe 1	Reagent	0 MIN	Ambient
<b>3</b>	<b>miR Probe 1</b>	<b>Reagent</b>	<b>120 MIN</b>	<b>37°C</b>
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	0 MIN	Ambient
14	*Bond Wash Solution	Wash	0 MIN	Ambient
15	miRNAscope Amp 1	Reagent	1 MIN	42°C
16	miRNAscope 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
28	*Bond Wash Solution	Wash	0 MIN	Ambient
29	*Bond Wash Solution	Open Wash	0 MIN	Ambient
30	*Bond Wash Solution	Wash	0 MIN	Ambient
31	miRNAscope Amp 2	Reagent	1 MIN	42°C
32	miRNAscope Amp 2	Reagent	15 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

Step No.	Reagent	Step Type	Incubation Time	Temperature
36	*Bond Wash Solution	Wash	1 MIN	Ambient
37	*Bond Wash Solution	Wash	1 MIN	Ambient
38	*Bond Wash Solution	Wash	1 MIN	Ambient
39	*Bond Wash Solution	Open Wash	1 MIN	Ambient
40	*Bond Wash Solution	Wash	1 MIN	Ambient
41	miRNAscope Amp 3	Reagent	1 MIN	42°C
42	miRNAscope Amp 3	Reagent	30 MIN	42°C
43	*Bond Wash Solution	Wash	0 MIN	Ambient
44	*Bond Wash Solution	Wash	0 MIN	Ambient
45	*Bond Wash Solution	Wash	0 MIN	Ambient
46	*Bond Wash Solution	Wash	3 MIN	Ambient
47	*Bond Wash Solution	Wash	3 MIN	Ambient
48	*Bond Wash Solution	Wash	0 MIN	Ambient
49	*Bond Wash Solution	Wash	0 MIN	Ambient
50	*Bond Wash Solution	Wash	0 MIN	Ambient
51	*LS Rinse	Reagent	5 MIN	Ambient
52	*LS Rinse	Reagent	5 MIN	Ambient
53	*Bond Wash Solution	Wash	0 MIN	Ambient
54	*Bond Wash Solution	Wash	1 MIN	Ambient
55	*Bond Wash Solution	Open Wash	1 MIN	Ambient
56	*Bond Wash Solution	Wash	1 MIN	Ambient
57	miRNAscope Amp 4-Red	Reagent	1 MIN	42°C
58	miRNAscope Amp 4-Red	Reagent	15 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	1 MIN	Ambient
63	*Bond Wash Solution	Wash	1 MIN	Ambient
64	*Bond Wash Solution	Wash	1 MIN	Ambient
65	*Bond Wash Solution	Open Wash	1 MIN	Ambient
66	*Bond Wash Solution	Wash	1 MIN	Ambient
67	miRNAscope Amp 5-Red	Reagent	1 MIN	Ambient
68	miRNAscope Amp 5-Red	Reagent	15 MIN	Ambient
69	*Bond Wash Solution	Wash	0 MIN	Ambient
70	*Bond Wash Solution	Wash	0 MIN	Ambient
71	*Bond Wash Solution	Wash	0 MIN	Ambient
72	*Bond Wash Solution	Wash	1 MIN	Ambient
73	*Bond Wash Solution	Wash	1 MIN	Ambient
74	*Bond Wash Solution	Wash	1 MIN	Ambient

Step No.	Reagent	Step Type	Incubation Time	Temperature
75	*Bond Wash Solution	Wash	1 MIN	Ambient
76	*Bond Wash Solution	Wash	1 MIN	Ambient
77	miRNAscope Amp 6-Red	Reagent	1 MIN	Ambient
78	miRNAscope Amp 6-Red	Reagent	15 MIN	Ambient
79	*Bond Wash Solution	Wash	0 MIN	Ambient
80	*Bond Wash Solution	Wash	0 MIN	Ambient
81	*Bond Wash Solution	Wash	0 MIN	Ambient
82	*Bond Wash Solution	Wash	1 MIN	Ambient
83	*Bond Wash Solution	Wash	1 MIN	Ambient
84	*Bond Wash Solution	Wash	1 MIN	Ambient
85	*Bond Wash Solution	Wash	1 MIN	Ambient
86	*Bond Wash Solution	Wash	1 MIN	Ambient
87	*LS Rinse	Reagent	5 MIN	Ambient
88	*LS Rinse	Reagent	5 MIN	Ambient
89	*Mixed Red Refine	Reagent	1 MIN	Ambient
90	*Mixed Red Refine	Reagent	10 MIN	Ambient
91	*De-ionized Water	Wash	0 MIN	Ambient
92	*De-ionized Water	Wash	0 MIN	Ambient
93	*De-ionized Water	Wash	0 MIN	Ambient
94	*De-ionized Water	Wash	0 MIN	Ambient
95	*De-ionized Water	Wash	0 MIN	Ambient
96	*De-ionized Water	Wash	0 MIN	Ambient
97	*De-ionized Water	Wash	0 MIN	Ambient
98	*Hematoxylin	Reagent	5 MIN	Ambient
99	*De-ionized Water	Wash	0 MIN	Ambient
100	*De-ionized Water	Wash	0 MIN	Ambient
101	*De-ionized Water	Wash	0 MIN	Ambient
102	*De-ionized Water	Wash	0 MIN	Ambient
103	*De-ionized Water	Wash	0 MIN	Ambient
104	*De-ionized Water	Wash	0 MIN	Ambient
105	†ACD Blue (optional)	Reagent	2 MIN	Ambient
106	†De-ionized Water (Optional)	Wash	0 MIN	Ambient
107	†De-ionized Water (Optional)	Wash	0 MIN	Ambient
108	†De-ionized Water (Optional)	Wash	0 MIN	Ambient
109	†De-ionized Water (Optional)	Wash	0 MIN	Ambient

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

† Optional bluing steps performed on the instrument. Bluing can also be performed offline using other types of bluing reagents.

7. Compare and confirm the on-screen protocol with the preceding table.
8. Select **Preferred** in the bottom right corner of the window.

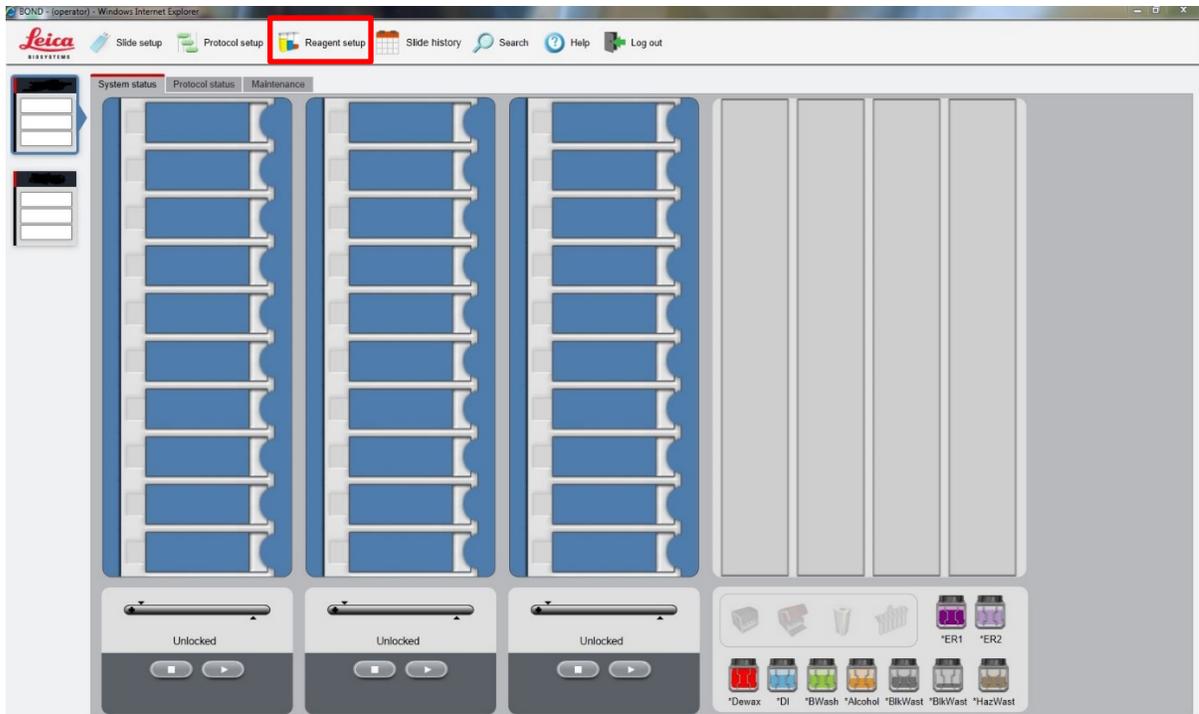
9. Select **Save**.
10. Click on **Next** to proceed. Ignore any pop-ups that may appear on the screen.

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

## Register the mock probe

Create a mock probe in the reagent set up.

1. Click on the **Reagent setup** icon.



2. Select **Add**.

3. Enter the mock probe name 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 miR ISH Red P1** 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.
10. Select **Preferred**, then select **Save**.

### Add reagent

Name:

Abbreviated name:

Type: Probe RNA | ↕

Supplier:

Single/double stain Single/Sequential DS | ↕

Single
First
Second

Default staining protocol: ACD miR ISH Red P1 | ↕

Default HIER protocol: \*ACD HIER 15 min with ER2 (95) | ↕

Default enzyme protocol: \*ACD 15 min Protease | ↕

Default denaturation protocol: \* - - - | ↕

Default hybridization protocol: ACD 1min Hybridization | ↕

Compatible bulks:

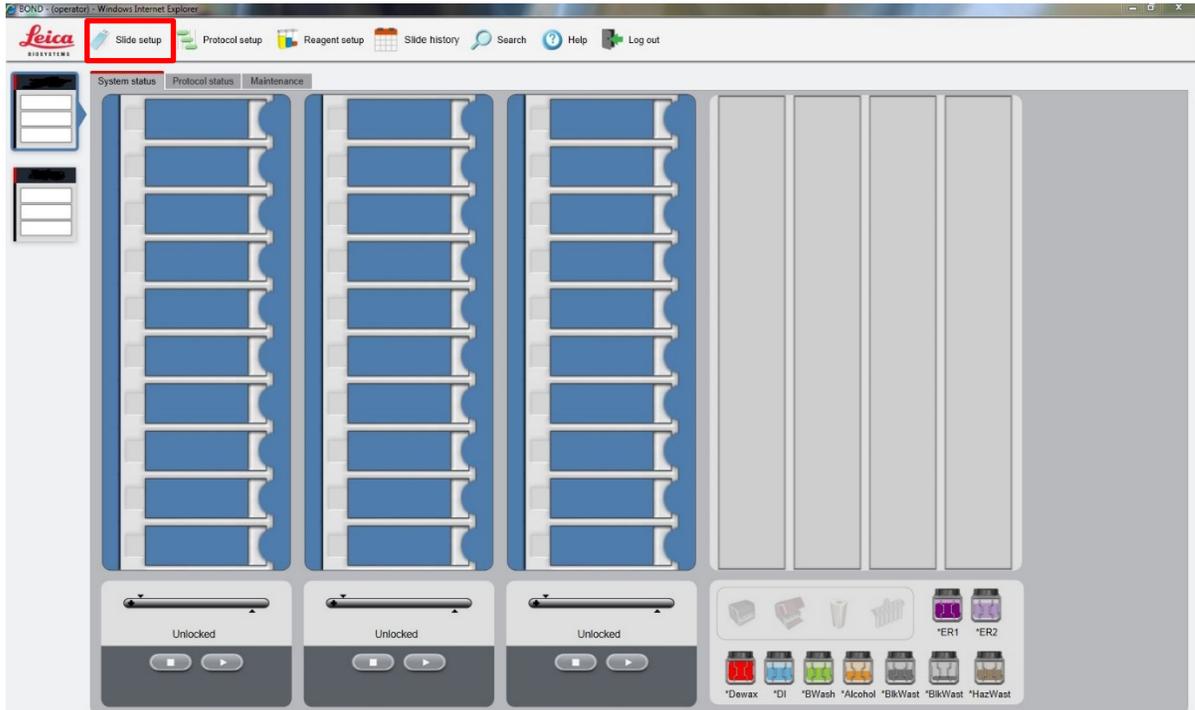
\*BWash

Preferred
  Hazardous

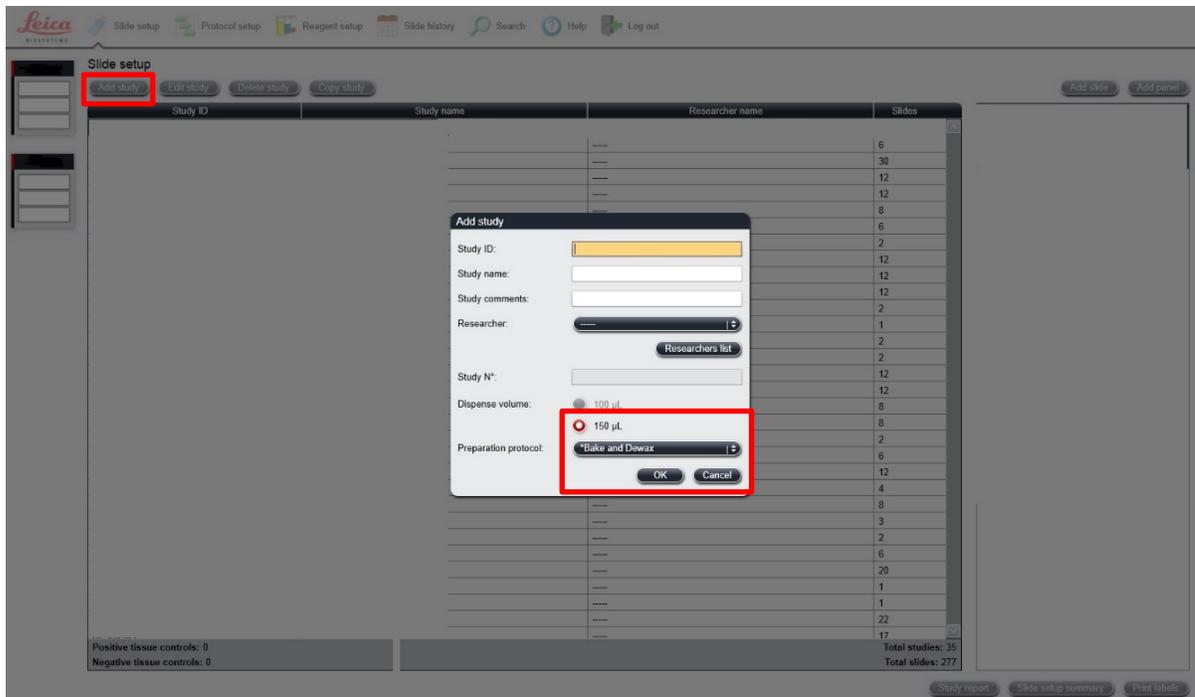
Save
Cancel

## Set up a study

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

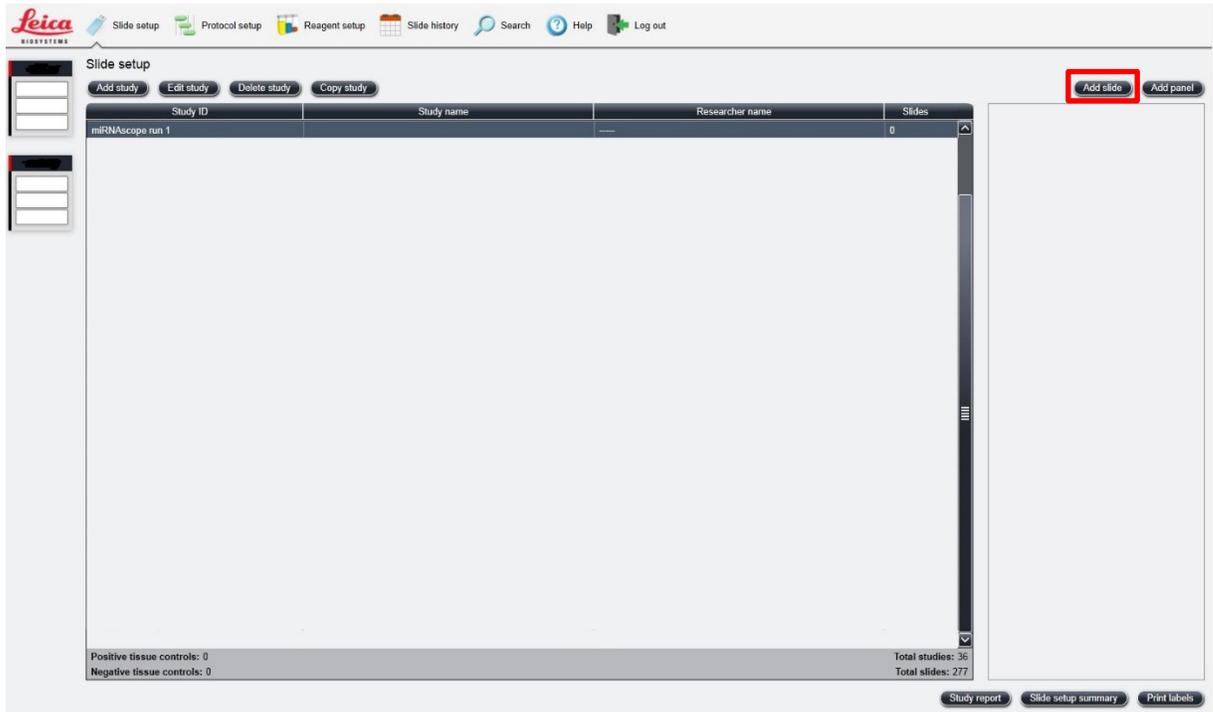


2. Select **Add study** and enter a name in the Study ID field (keep the Dispense volume at 150  $\mu$ l as shown).



3. For FFPE tissues, select **\*Bake and Dewax** as the Preparation protocol. For fresh-frozen tissues, select **\*---** instead of **\*Bake and Dewax**.
4. Select **OK**.

5. Select **Add slide** to assign a protocol to each slide.



6. Enter the tissue type and probe name in the Comments field.
7. Keep **Single** as default from the Staining mode drop down menu.
8. Select **ISH** for Process and **Mock Probe (ACD)** from the Marker drop down menu.
9. In the **Protocols** tab, do the following:
  - a. For each distinct probe, select a different protocol from the Staining drop down menu (for example, ACD miR Red ISH P1).
  - b. For FFPE tissues, select the protocol **\*Bake and Dewax** from the Preparation drop down menu. For fresh-frozen tissues, select **\*----** instead of **\*Bake and Dewax**.
  - c. For FFPE tissues, select **\*ACD HIER 15 min with ER2 (95)** as the HIER protocol. For fresh-frozen tissues, select **\*----**.
- Note:** HIER protocol time and temperature varies depending on the tissue type.
- d. For FFPE tissues, select **\*ACD 15 min Protease** for Enzyme, or the appropriate enzyme protocol for your tissue (see **Appendix B** on page 50 to edit the protease protocol).
- e. Select **ACD 1 min Hybridization** for Hybridization.
10. Select **Add slide** for each target probe and for each of the slides used in the run.
11. After adding all the slides to the study, select **Close** to return to the Slide setup screen.

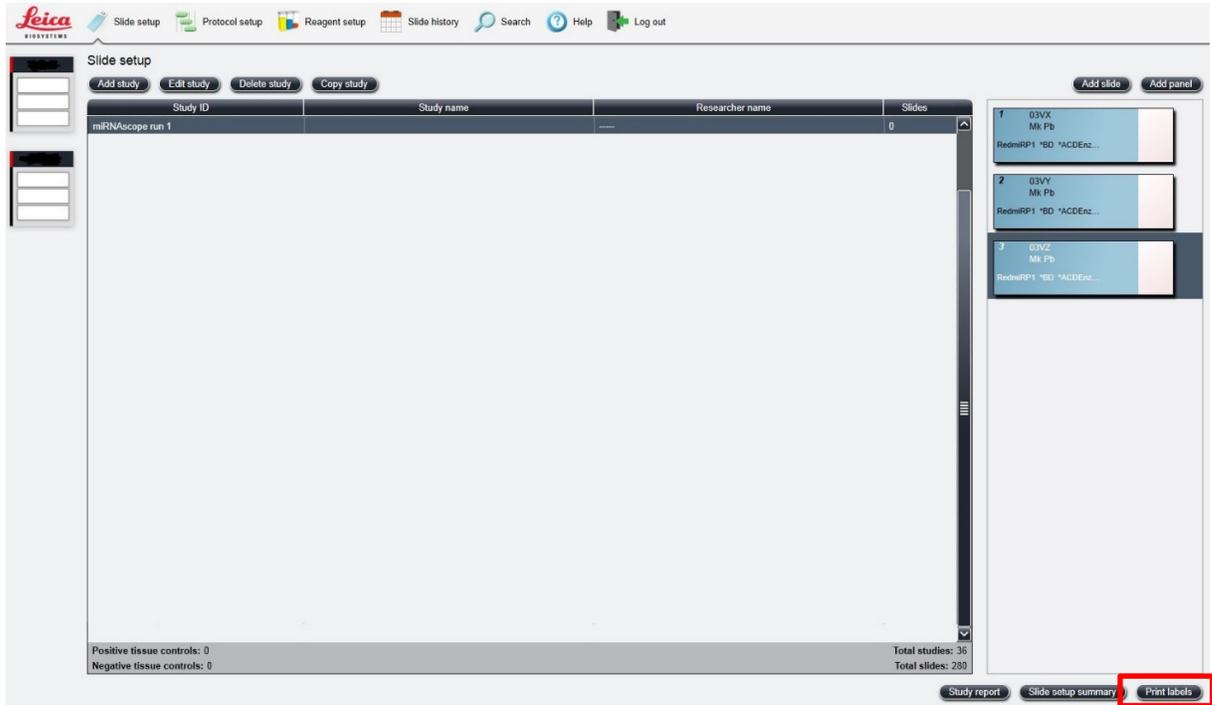


The screenshot shows the 'Add slide' configuration window. It includes the following sections:

- Study Information:** Slide ID (empty), Study N° (394), Study name (empty), Study comments (empty), Study ID (miRNAscope run 1), and Comments (tissue type probe 1).
- Tissue type:** Radio buttons for Test tissue (selected), Negative tissue, and Positive tissue.
- Dispense volume:** Radio buttons for 100 µL and 150 µL (selected).
- Staining mode:** Two dropdown menus, 'Single' and 'Routine', both set to 'Single'.
- Process:** Radio buttons for IHC and ISH (selected).
- Marker:** A dropdown menu set to 'Mock Probe (ACD)'.
- Protocols:** A section with dropdown menus for:
  - Staining: ACD miR ISH Red P1
  - Preparation: \*Bake and Dewax
  - HIER: \*ACD HIER 15 min with ER2 (95)
  - Enzyme: \*ACD 15 min Protease
  - Denaturation: \*----
  - Hybridization: ACD 1min Hybridization

At the bottom right, there are 'Add slide' and 'Close' buttons.

12. Select **Print labels** to print barcodes to attach to the slides.



13. Proceed to **Chapter 6. Run the miRNAscope™ LS Assay** on page 41.

# 5

## Chapter 5. Set Up Software Version 6.0

Use the instructions in this chapter to set up the miRNAscope™ LS Assay using software version 6.0. To set up the assay using software version 5.2, see **Chapter 4** on page 12.

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**IMPORTANT!** BXD20 or higher is required to run the following setup on software version 6.0. Please contact your Leica FAS to upgrade to BXD20 before starting the assay.

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

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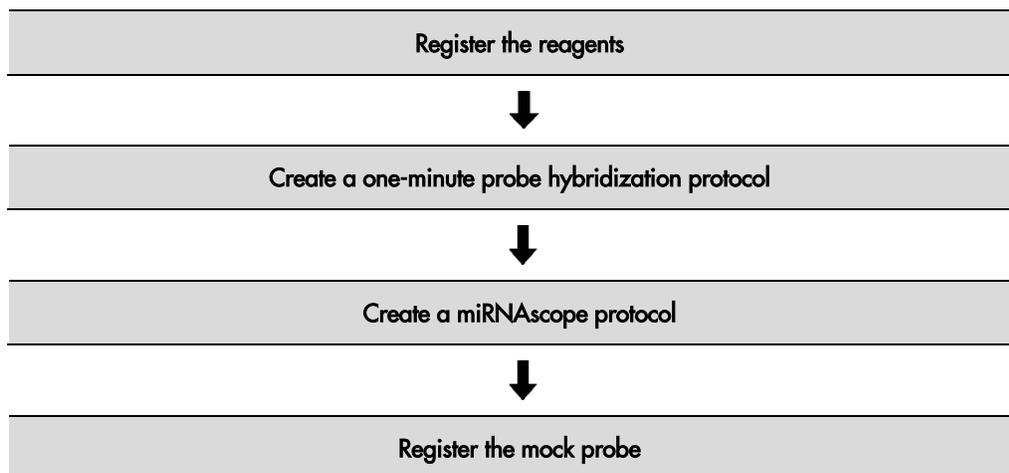


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**IMPORTANT!** Make sure you have software version 5.2 or 6.0 before setting up the assay. The miRNAscope™ LS Assay is not supported by software version 4.0.

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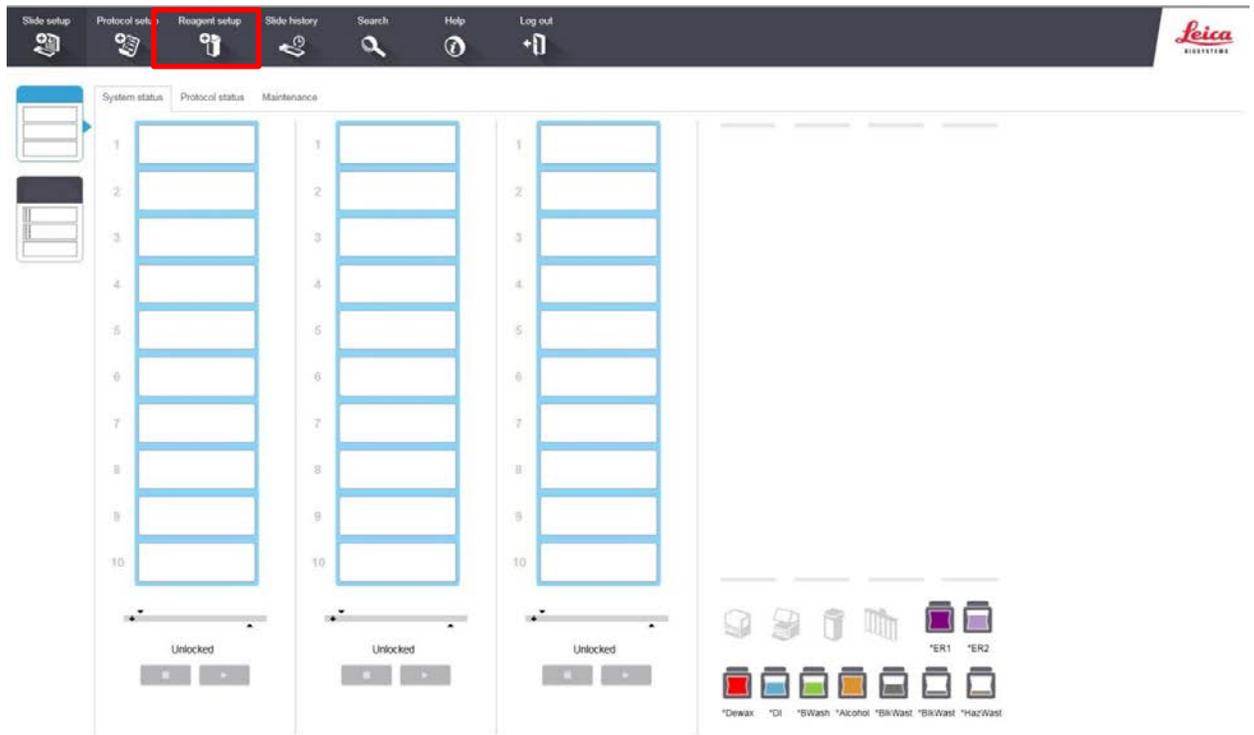
### Workflow



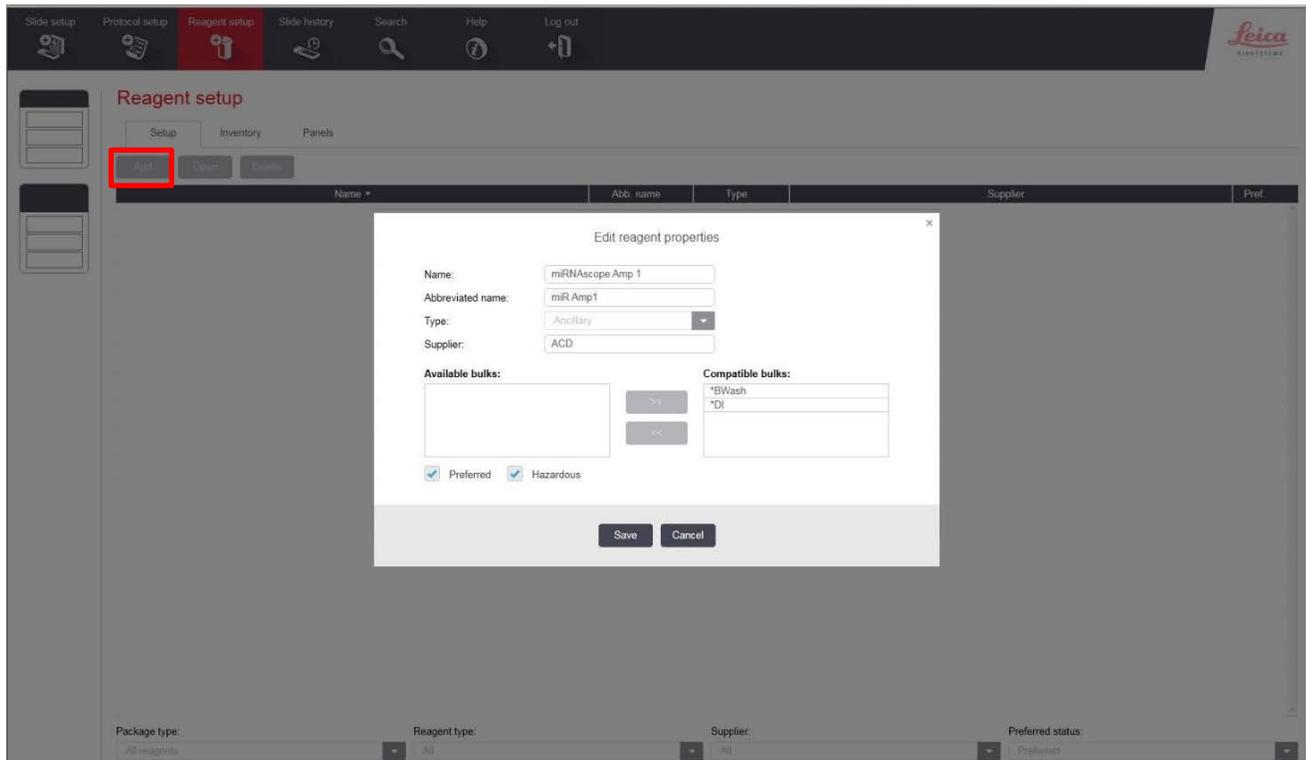
## Register the reagents

Register the amplification reagents (for example, ACD miRNAscope Amp 1) as ancillary reagents.

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



2. Select **Add** to enter reagent information.



- Enter a name (for example, **miRNAscope Amp 1**) in the Name text box.

- Enter **miR Amp 1** (for example) in the Abbreviated name text box.
- Select **Ancillary** in the Type drop-down menu.
- Enter **ACD** in the Supplier text box.
- For miRNAscope™ Amp 1 and miRNAscope™ 3 only, check both the **Preferred** and **Hazardous** boxes.
- Select **Save**.
- Repeat Steps 2–7 to register the rest of the reagents using the container names in the following table:

Reagent	Name	Abbreviated Name
miRNAscope™ LS Amp 1	miRNAscope Amp 1	miR Amp 1
miRNAscope™ LS Amp 2	miRNAscope Amp 2	miR Amp 2
miRNAscope™ LS Amp 3	miRNAscope Amp 3	miR Amp 3
miRNAscope™ LS Amp 4-Red	miRNAscope Amp 4 Red	miR Amp 4
miRNAscope™ LS Amp 5-Red	miRNAscope Amp 5 Red	miR Amp 5
miRNAscope™ LS Amp 6-Red	miRNAscope Amp 6 Red	miR Amp 6

**Note:** Complete these steps only once for each reagent. Bluing is optional and can be completed off the instrument.

## Create the miRNA probe hybridization protocol

1. In the Protocol setup screen, select **Prestaining** from the Protocol group menu and **ISH Hybridization** from the Protocol type menu.
2. Highlight the **\*RNAscope 2.5 LSx Hybridization** protocol. Select **Copy**.

The screenshot shows the 'Protocol setup' interface. At the top, there is a navigation bar with icons for Slide setup, Protocol setup (highlighted in red), Reagent setup, Slide history, Search, Help, and Log out. The Leica logo is in the top right corner. Below the navigation bar, there are buttons for Copy (highlighted with a red box), Open, Delete, and Report. A table lists protocols, with the first row highlighted in blue:

Protocol name	Protocol type	Description	Modified by	Mod. date	Prot.
*RNAscope 2.5 LSx Hybridization	ISH hybridization	RNAscope 2.5 LSx 2 hour Hybridization	Leica	7/25/2019	✓

At the bottom of the screen, there are several dropdown menus: Protocol group (set to 'Prestaining', highlighted with a red box), Protocol type (set to 'ISH hybridization', highlighted with a red box), Staining status (set to 'All'), Protocol origin (set to 'All'), and Preferred status (set to 'Preferred').

3. Change the Name to **ACD miRNAscope 37C**, the Abbreviated Name to **ACDmiR37**, and the Description to **ACD miRNAscope hybridization 37C**.

New protocol properties

Name: ACD miRNAscope 37C  
 Abbreviated name: ACDmiR37  
 Description: ACD miRNAscope Hybridization 37C

Preferred

BOND RX Import protocol Protocol type: ISH hybridization

Step N°	Wash	Reagent	Supplier	Ambient	Temperature	Inc. (min)	Dispense type
1		*No Reagent	Leica Microsystems		37	120.00	Intermediate

Show wash steps

**Save** **Cancel**

Protocol group: Prestaining Protocol type: ISH hybridization Staining status: All Protocol origin: All Preferred status: Preferred

4. Change the temperature to **37°C**.

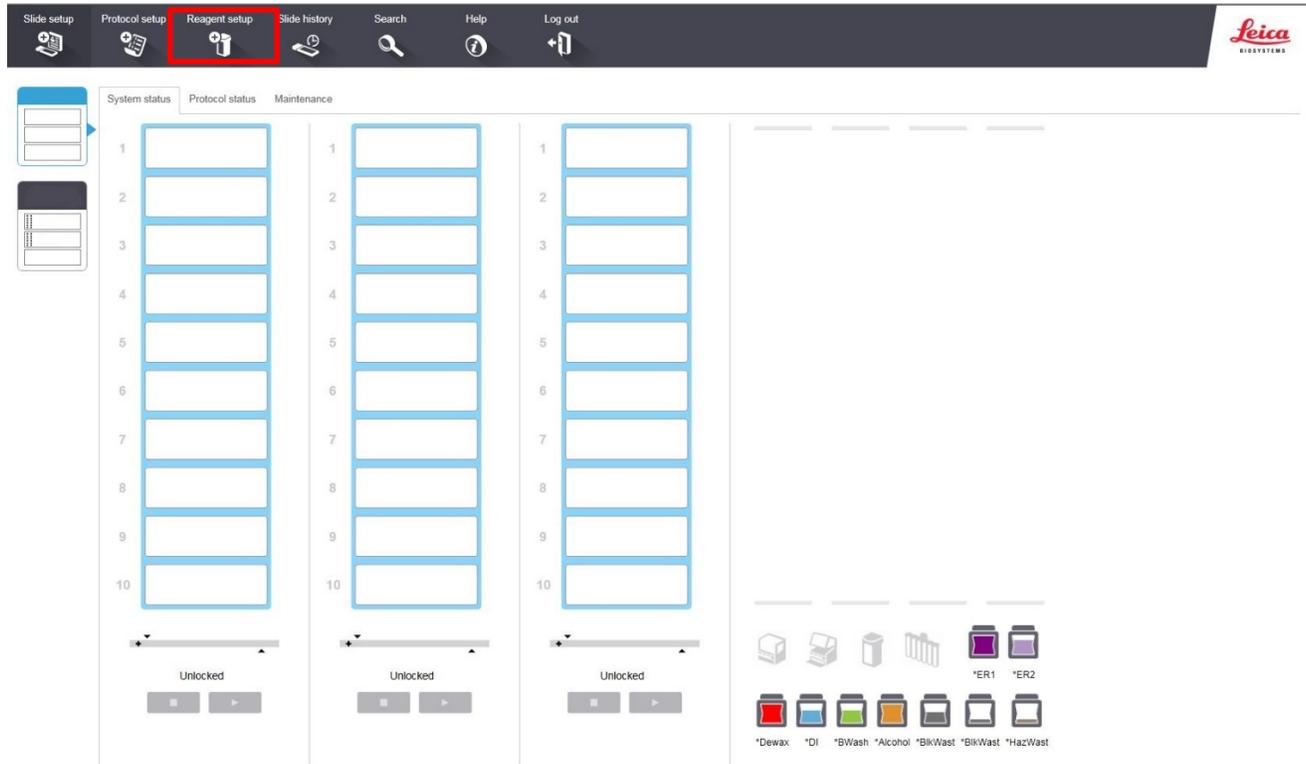
**IMPORTANT!** To assure the success of the assay, the temperature must be **37°C**.

5. Select **Save**.

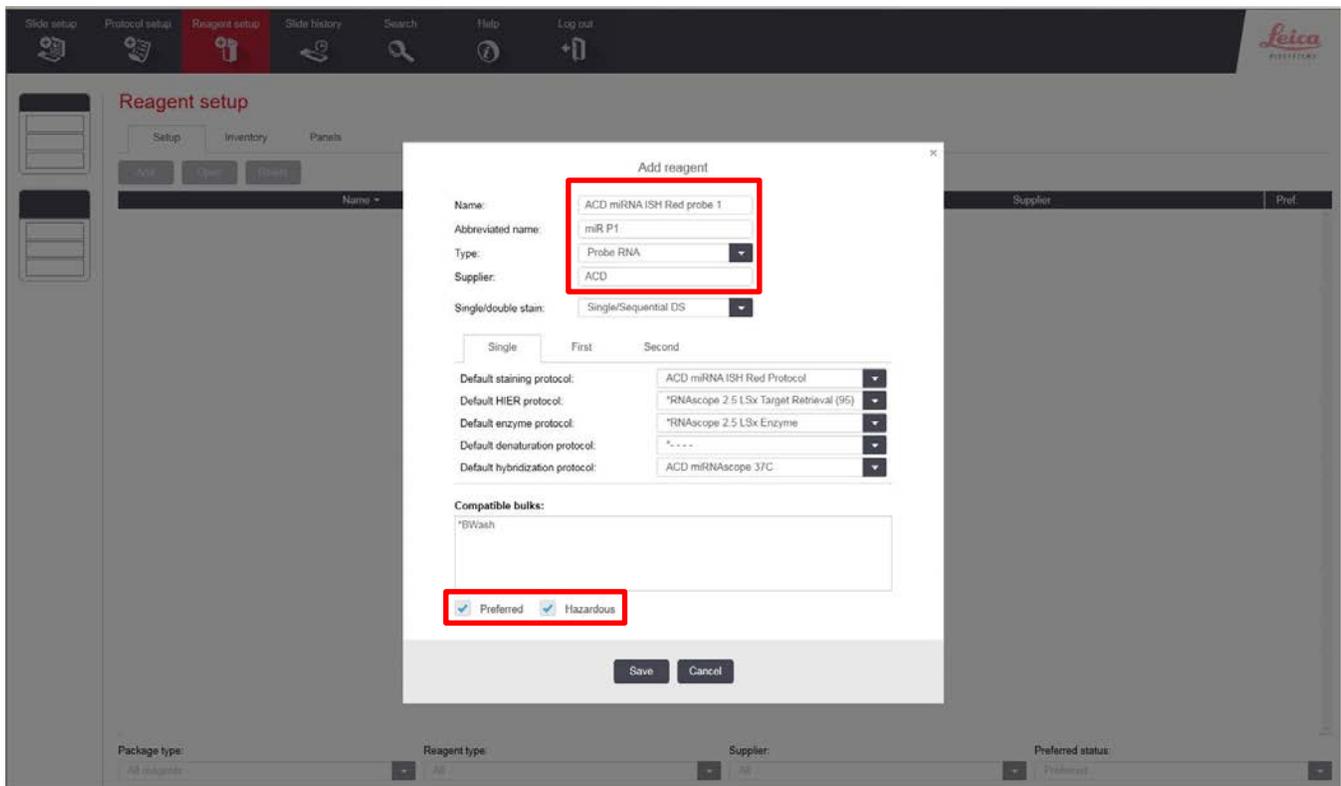
## Register the probes

If you have software version 6.0, you can register the probes as probes. Contact your ACD Field Application Specialist (FAS) to assist you.

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



2. Select **Add** to enter reagent information.



3. Enter the probe name (for example, ACD miRNA ISH Red probe 1) in the Name text box.

**Note:** Do not reuse the names of any Ancillary probes created for BXD11.

4. Enter **miR P1** (for example) in the Abbreviated name text box.

5. Select **Probe RNA** in the Type drop-down menu.
6. Enter **ACD** in the Supplier text box.
7. Select ACD miRNA ISH Red Protocol as the Default Staining protocol
8. Select **\*RNAscope 2.5 LSx Target Retrieval (95)** as the Default HIER protocol.
9. Select **\*RNAscope 2.5 LSx Enzyme** as the Default enzyme protocol.
10. Leave the Default denaturation protocol blank.
11. Select **ACD miRNAscope 37C** as the Default hybridization protocol.
12. Check both the **Preferred** and **Hazardous** boxes.
13. Select **Save**.

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

## Create the staining protocol

For software version 6.0, you can use the same staining protocol for all miRNAscope™ probes.

1. In the Protocol setup screen, select **Staining** from the Protocol group menu.
2. Highlight the **\*ACD Red ISH Protocol** protocol. Select **Copy**.

The screenshot shows the 'Protocol setup' interface. At the top, there are navigation tabs: Slide setup, Protocol setup (active), Reagent setup, Slide history, Search, Help, and Log out. The Leica logo is in the top right. Below the tabs, there are buttons for Copy, Open, Delete, and Report. The 'Copy' button is highlighted with a red box. A table lists protocols:

Protocol name	Protocol type	Description	Modified by	Mod. date	Pref.
*ACD Red ISH Protocol	ISH detection	ACD RNAscope Red ISH protocol	Leica	7/25/2019	✓

At the bottom, there are filter dropdowns: Protocol group (highlighted with a red box and set to 'Staining'), Protocol type (All), Staining status (All), Protocol origin (All), and Preferred status (Preferred).

3. Change the protocol name to **ACD miR ISH Red Protocol** in the Name text box, **RedmiR** in the Abbreviated name text box, and **ACD miRNAscope ISH Red protocol** in the Description text box.

**New protocol properties**

Name: ACD miRNA ISH Red Protocol  
 Abbreviated name: RedmiR  
 Description: ACD miRNAAscope ISH Red protocol  
 Staining method:  Single  First  Second  Preferred

BOND RX Import protocol Protocol type: ISH detection

Preferred detection system: Bond Polymer Refine Red Detection

Step N°	Wash	Reagent	Supplier	Ambient	Temperature	Inc. (min)	Dispense type
1		miRNAAscope Amp 1	ACD		42	1:00	150 µL
2		miRNAAscope Amp 1	ACD		42	30:00	150 µL
17		miRNAAscope Amp 2	ACD		42	1:00	150 µL
18		miRNAAscope Amp 2	ACD		42	15:00	150 µL
27		miRNAAscope Amp 3	ACD		42	1:00	150 µL
29		miRNAAscope Amp 3	ACD		42	30:00	150 µL
30	<input checked="" type="checkbox"/>	*Bond Wash Solution	Leica Microsystems	<input checked="" type="checkbox"/>		0:00	150 µL
31	<input checked="" type="checkbox"/>	*Bond Wash Solution	Leica Microsystems	<input checked="" type="checkbox"/>		0:00	150 µL
32	<input checked="" type="checkbox"/>	*Bond Wash Solution	Leica Microsystems	<input checked="" type="checkbox"/>		3:00	150 µL

Show wash steps Insert wash | Insert reagent | Delete step

Save Cancel

**New protocol properties**

Name: ACD miRNA ISH Red Protocol  
 Abbreviated name: RedmiR  
 Description: ACD miRNAAscope ISH Red protocol  
 Staining method:  Single  First  Second  Preferred

BOND RX Import protocol Protocol type: ISH detection

Preferred detection system: Bond Polymer Refine Red Detection

Step N°	Wash	Reagent	Supplier	Ambient	Temperature	Inc. (min)	Dispense type
37		miRNAAscope Amp 4 Red	ACD		42	1:00	150 µL
38		miRNAAscope Amp 4 Red	ACD		42	15:00	150 µL
47		miRNAAscope Amp 5 Red	ACD	<input checked="" type="checkbox"/>		1:00	150 µL
48		miRNAAscope Amp 5 Red	ACD	<input checked="" type="checkbox"/>		15:00	150 µL
57		miRNAAscope Amp 6 Red	ACD	<input checked="" type="checkbox"/>		1:00	150 µL
58		miRNAAscope Amp 6 Red	ACD	<input checked="" type="checkbox"/>		15:00	150 µL
65	<input checked="" type="checkbox"/>	*Bond Wash Solution	Leica Microsystems	<input checked="" type="checkbox"/>		1:00	150 µL
66	<input checked="" type="checkbox"/>	*Bond Wash Solution	Leica Microsystems	<input checked="" type="checkbox"/>		1:00	150 µL

Show wash steps Insert wash | Insert reagent | Delete step

Save Cancel

**Note:** The preceding two figures display all reagent steps.  
 4. Click on **Show wash steps** to also view the wash steps.

5. To edit each step, highlight and select it. Set up each step, including wash steps, according to the following table.

Step No.	Reagent	Step Type	Incubation Time	Temperature
1	miRNAscope Amp 1	Reagent	1 MIN	42°C
2	miRNAscope Amp 1	Reagent	30 MIN	42°C
3	*Bond Wash Solution	Wash	0 MIN	Ambient
4	*Bond Wash Solution	Wash	0 MIN	Ambient
5	*Bond Wash Solution	Wash	0 MIN	Ambient
6	*Bond Wash Solution	Wash	3 MIN	Ambient
7	*Bond Wash Solution	Wash	3 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	*LS Rinse	Reagent	5 MIN	Ambient
12	*LS Rinse	Reagent	5 MIN	Ambient
13	*Bond Wash Solution	Wash	0 MIN	Ambient
14	*Bond Wash Solution	Wash	0 MIN	Ambient
15	*Bond Wash Solution	Open Wash	0 MIN	Ambient
16	*Bond Wash Solution	Wash	0 MIN	Ambient
17	miRNAscope Amp 2	Reagent	1 MIN	42°C
18	miRNAscope Amp 2	Reagent	15 MIN	42°C
19	*Bond Wash Solution	Wash	0 MIN	Ambient
20	*Bond Wash Solution	Wash	0 MIN	Ambient
21	*Bond Wash Solution	Wash	0 MIN	Ambient
22	*Bond Wash Solution	Wash	1 MIN	Ambient
23	*Bond Wash Solution	Wash	1 MIN	Ambient
24	*Bond Wash Solution	Wash	1 MIN	Ambient
25	*Bond Wash Solution	Open Wash	1 MIN	Ambient
26	*Bond Wash Solution	Wash	1 MIN	Ambient
27	miRNAscope Amp 3	Reagent	1 MIN	42°C
28	miRNAscope Amp 3	Reagent	30 MIN	42°C
29	*Bond Wash Solution	Wash	0 MIN	Ambient
30	*Bond Wash Solution	Wash	0 MIN	Ambient
31	*Bond Wash Solution	Wash	0 MIN	Ambient
32	*Bond Wash Solution	Wash	3 MIN	Ambient
33	*Bond Wash Solution	Wash	3 MIN	Ambient
34	*Bond Wash Solution	Wash	1 MIN	Ambient
35	*Bond Wash Solution	Open Wash	1 MIN	Ambient
36	*Bond Wash Solution	Wash	0 MIN	Ambient
37	miRNAscope Amp 4-Red	Reagent	1 MIN	42°C

Step No.	Reagent	Step Type	Incubation Time	Temperature
38	miRNAscope Amp 4–Red	Reagent	15 MIN	42°C
39	*Bond Wash Solution	Wash	0 MIN	Ambient
40	*Bond Wash Solution	Wash	0 MIN	Ambient
41	*Bond Wash Solution	Wash	0 MIN	Ambient
42	*Bond Wash Solution	Wash	1 MIN	Ambient
43	*Bond Wash Solution	Wash	1 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	miRNAscope Amp 5–Red	Reagent	1 MIN	Ambient
48	miRNAscope Amp 5–Red	Reagent	15 MIN	Ambient
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	miRNAscope Amp 6–Red	Reagent	1 MIN	Ambient
58	miRNAscope Amp 6–Red	Reagent	15 MIN	Ambient
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	1 MIN	Ambient
63	*Bond Wash Solution	Wash	1 MIN	Ambient
64	*Bond Wash Solution	Wash	1 MIN	Ambient
65	*Bond Wash Solution	Wash	1 MIN	Ambient
66	*Bond Wash Solution	Wash	1 MIN	Ambient
67	*LS Rinse	Reagent	5 MIN	Ambient
68	*LS Rinse	Reagent	5 MIN	Ambient
69	*Mixed Red Refine	Reagent	1 MIN	Ambient
70	*Mixed Red Refine	Reagent	10 MIN	Ambient
71	*De-ionized Water	Wash	0 MIN	Ambient
72	*De-ionized Water	Wash	0 MIN	Ambient
73	*De-ionized Water	Wash	0 MIN	Ambient
74	*De-ionized Water	Wash	0 MIN	Ambient
75	*De-ionized Water	Wash	0 MIN	Ambient
76	*De-ionized Water	Wash	0 MIN	Ambient

Step No.	Reagent	Step Type	Incubation Time	Temperature
77	*Hematoxylin	Reagent	5 MIN	Ambient
78	*De-ionized Water	Wash	0 MIN	Ambient
79	*De-ionized Water	Wash	0 MIN	Ambient
80	*De-ionized Water	Wash	0 MIN	Ambient
81	*De-ionized Water	Wash	0 MIN	Ambient
82	†ACD Blue (Optional)	Reagent	2 MIN	Ambient
83	†De-ionized Water (Optional)	Wash	0 MIN	Ambient
84	†De-ionized Water (Optional)	Wash	0 MIN	Ambient
85	†De-ionized Water (Optional)	Wash	0 MIN	Ambient
86	†De-ionized Water (Optional)	Wash	0 MIN	Ambient

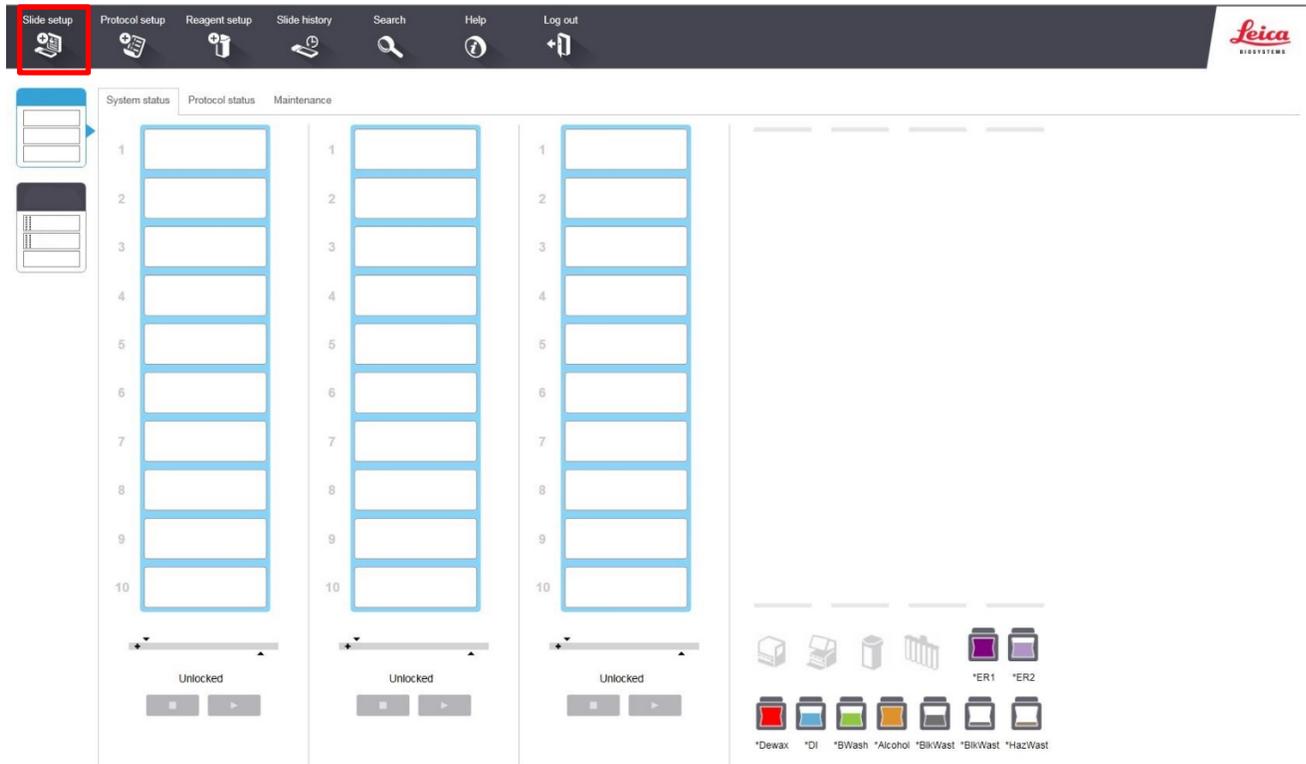
\* Indicates step is hard-coded in the software by Leica Biosystems.

† Optional bluing steps performed on the instrument. Bluing can also be performed offline using other types of bluing reagents.

6. Compare and confirm the on-screen protocol with the preceding table.
7. Select **Preferred** in the bottom right corner of the window.
8. Select **Save**.
9. Click on **Next** to proceed. Ignore any pop-ups that may appear on the screen.

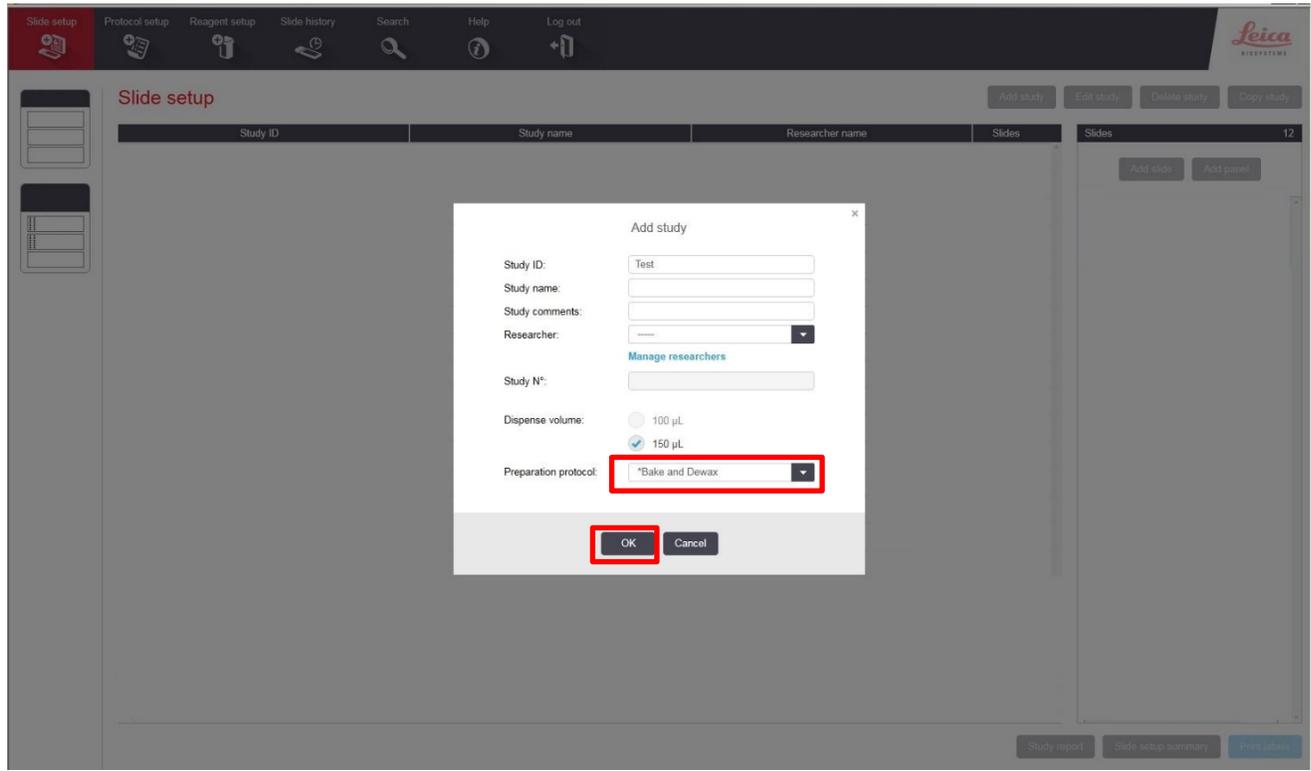
## Set up a study

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

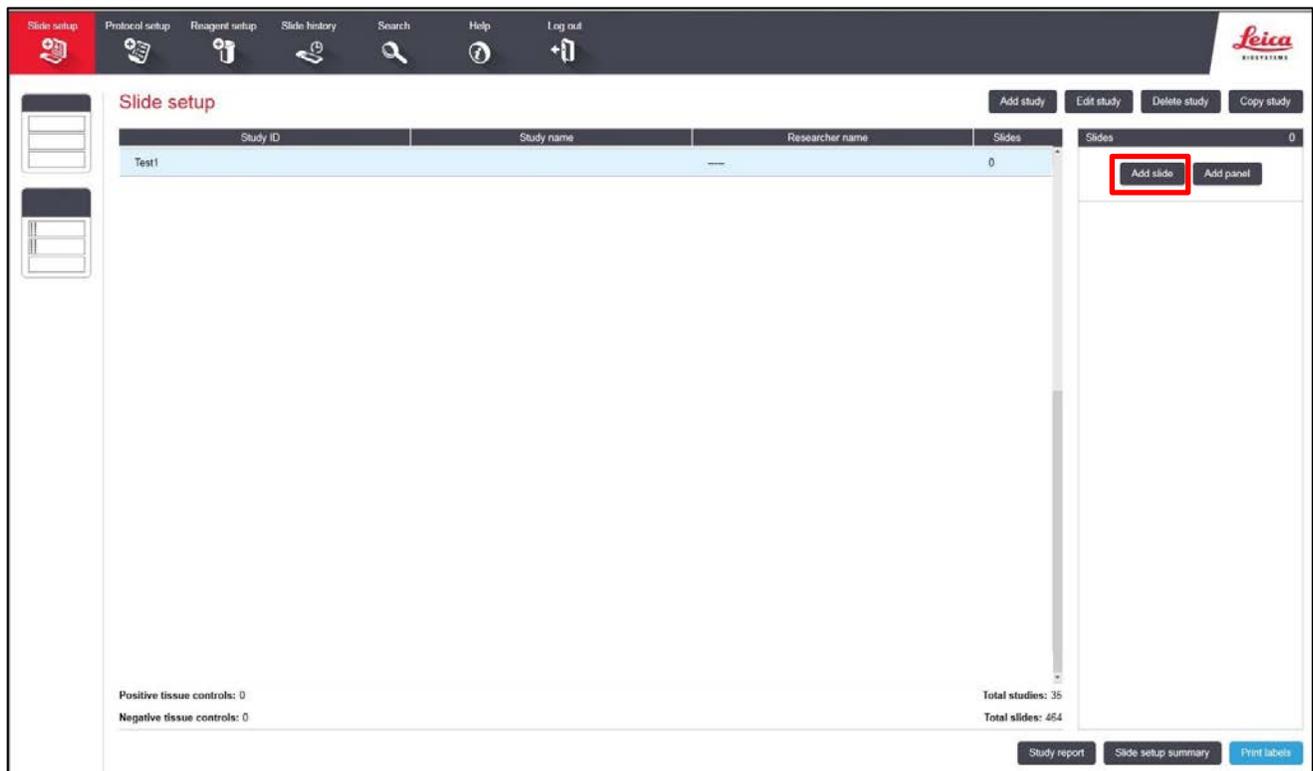


2. Select **Add study** and enter a name in the Study ID field (keep the Dispense volume at 150 µl as shown).

- For FFPE tissues, select **\*Bake and Dewax** as the Preparation protocol. For fresh frozen tissues, select \*--- instead of \*Bake and Dewax.
- Select **OK**.



- Select **Add slide** to assign a protocol to each slide.



6. Enter the tissue type and probe name in the Comments field.
7. Keep **Single** as default from the Staining mode drop down menu.
8. Select **ISH** for Process and **ACD miRNA ISH Red probe 1** from the Marker drop down menu.
9. In the **Protocols** tab, do the following:
  - a. Select the **ACD miRNA ISH Red Protocol** from the Staining drop down menu.
  - b. For FFPE tissues, select the protocol **\*Bake and Dewax** from the Preparation drop down menu. For fresh-frozen tissues, select **\*----** instead of **\*Bake and Dewax**.
  - c. For FFPE tissues, select **\*RNAscope 2.5 LSx Target Retrieval (95)** as the HIER protocol. For fresh-frozen tissues, select **\*----**.

**Note:** HIER protocol time and temperature varies depending on the tissue type.

- d. For FFPE tissues, select **\*RNAscope 2.5 LSx Enzyme** for Enzyme. For fresh-frozen tissue, select **\*RNAscope 2.5 LSx Enzyme IV 30min RT** (see **Appendix B** on page 50 to edit the protease protocol).
  - e. Select **\*RNAscope 2.5 LSx Probe Application** for Probe Application.
  - f. Leave Denaturation blank.
  - g. Select **ACD miRNAscope 37C** for Hybridization.
  - h. Select **\*RNAscope 2.5 LSx Probe Removal** for Probe Removal.
  - i. Select **OK**.
10. Select **Add slide** for each target probe and for each of the slides used in the run.
  11. After adding all the slides to the study, select **Close** to return to the Slide setup screen.

12. Select **Print labels** to print barcodes to attach to the slides.

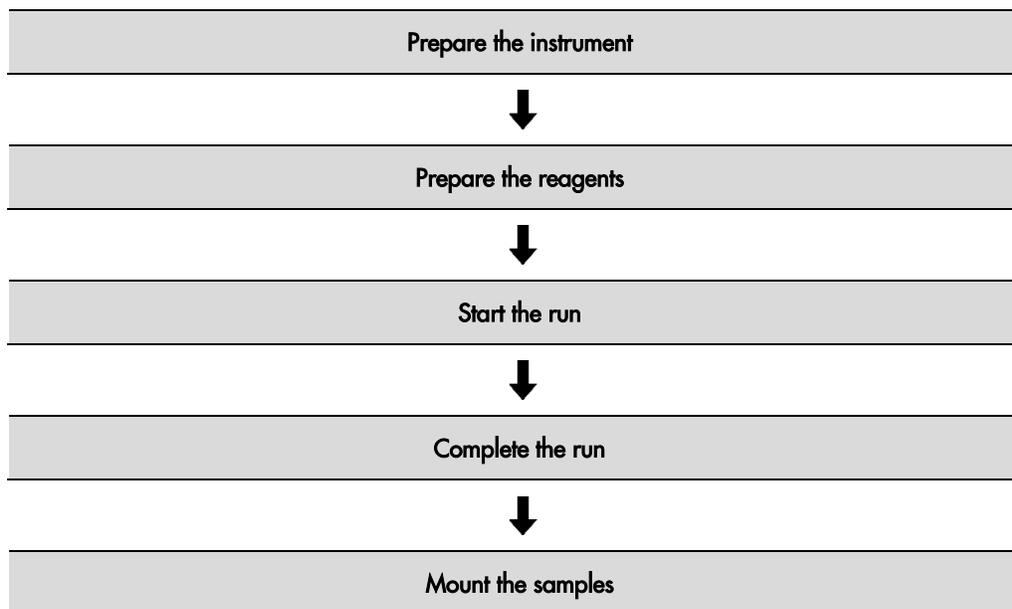
The screenshot shows the 'Slide setup' interface in the Leica Biosystems software. The top navigation bar includes 'Slide setup', 'Protocol setup', 'Reagent setup', 'Slide history', 'Search', 'Help', and 'Log out'. The main area is titled 'Slide setup' and contains a table with columns for 'Study ID', 'Study name', 'Researcher name', and 'Slides'. A single row is visible with 'Test1' in the 'Study ID' column and '1' in the 'Slides' column. To the right of the table is a 'Slides' panel with 'Add slide' and 'Add panel' buttons. Below the table, there are buttons for 'Study report', 'Slide setup summary', and 'Print labels', with the 'Print labels' button highlighted by a red box.

**IMPORTANT!** For optimal assay results, use this setup protocol.

# 6

## Chapter 6. Run the miRNAscope™ LS Assay

### Workflow



## Materials required

Materials provided by Advanced Cell Diagnostics	Materials provided by Leica Biosystems	Other materials and equipment
<ul style="list-style-type: none"> <li>miRNAscope™ LS Target Probe</li> <li>miRNAscope™ LS Positive Control Probe</li> <li>miRNAscope™ LS Negative Control Probe</li> <li>RNAscope® 2.5 LS Hydrogen Peroxide</li> <li>RNAscope® 2.5 LS Rinse</li> <li>RNAscope® LS Protease III</li> <li>miRNAscope™ LS Amp 1</li> <li>miRNAscope™ LS Amp 2</li> <li>miRNAscope™ LS Amp 3</li> <li>miRNAscope™ LS Amp 4–Red</li> <li>miRNAscope™ LS Amp 5–Red</li> <li>miRNAscope™ LS Amp 6–Red</li> <li>RNAscope® 2.5 LS Bluing Reagent (Optional)</li> </ul>	<ul style="list-style-type: none"> <li>Leica Biosystems' BOND RX System</li> <li>BOND 30 mL Open Containers</li> <li>BOND Universal Covertiles</li> <li>BOND Aspirating Probe Cleaning System</li> <li>BOND Mixing Stations</li> </ul> <p>LS Bulk Reagents</p> <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</li> <li>BOND Polymer Refine Red Detection and Hematoxylin</li> </ul>	<ul style="list-style-type: none"> <li>Distilled Water</li> <li>Tissue-Tek® Slide Rack</li> <li>Tissue-Tek® Staining Dishe</li> <li>Tissue-Tek® Clearing Agent Dishes</li> <li>Ammonium hydroxide, 28–30% (optional)</li> <li>Drying Oven</li> <li>EcoMount mounting media</li> <li>Cover Glass</li> </ul>

## Prepare the instrument

- 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 could 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.

- Use clean, dry covertiles for every run. Follow the instructions from Leica Biosystems to clean used covertiles with water, bleach, and ethanol. Air dry before reuse.
- Before starting a run, empty bulk waste containers. Discard waste according to all local, state/provincial and/or national regulations.

## Prepare the instrument reagents

- For version 5.2 software **ONLY**, label an empty BOND Open container **Mock Probe**, and fill it with 1X BOND Wash from Leica Biosystems.

**Note:** Follow the remaining steps for both version 5.2 and version 6.0 software.

- Carefully transfer all the RNAscope® and miRNAscope™ LS reagents into empty 30 mL BOND Open containers.
- Using the Leica barcode scanner, scan the barcode located on the front of the BOND Open container. A window will appear.
- From the drop-down menu, select the corresponding name of the reagent as shown in the following table under **Container name 5.2** or **Container name 6.0** depending on your software version:

Reagents	Container name 5.2	Container name 6.0
RNAscope® 2.5 LS Hydrogen Peroxide	*Open 0 Haz	*RNAscope 2.5 LSx H <sub>2</sub> O <sub>2</sub>
RNAscope® 2.5 LS Protease III	*ACD Enzyme	*RNAscope 2.5 LSx Enzyme

Reagents	Container name 5.2	Container name 6.0
RNAscope® 2.5 LS Rinse	*LS Rinse	*LS Rinse
miRNAscope™ LS Amp 1	miRNAscope Amp 1	miRNAscope Amp 1
miRNAscope™ LS Amp 2	miRNAscope Amp 2	miRNAscope Amp 2
miRNAscope™ LS Amp 3	miRNAscope Amp 3	miRNAscope Amp 3
miRNAscope™ LS Amp 4-Red	miRNAscope Amp 4 Red	miRNAscope Amp 4 Red
miRNAscope™ LS Amp 5-Red	miRNAscope Amp 5 Red	miRNAscope Amp 5 Red
miRNAscope™ LS Amp 6-Red	miRNAscope Amp 6 Red	miRNAscope Amp 6 Red
RNAscope® 2.5 Bluing Reagent	*ACD Blue (Optional)	*ACD Blue (Optional)
miRNAscope™ LS Target Probe	Variable (miR P1)	Variable (miR P1)
1X BOND Wash	Mock Probe	—

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

**Note:** RNAscope® 2.5 LS Rinse containers should be used for the entire 60 slides within a single kit.

**Note:** Leica BOND Red and Hematoxylin come in pre-filled Leica BOND RX containers.

5. Enter the RNAscope® 2.5 LS Reagent Kit or miRNAscope™ LS Reagent Kit lot number and the expiration date in their respective fields. Select **OK**.

## Start the run

1. Attach the barcodes to the slides, and add the slides to the slide tray with the label sides facing up.
2. Add a covertile on top of each slide, and verify placement and seating of each covertile.

**Note:** The rectangular-shaped neck of the covertile should fit into the groove of the slide tray.

3. Place the tray in the Leica BOND RX™, and press the button to load the tray onto the machine.
4. 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. Refer to **Troubleshooting** on page 46 if any issues occur.

---

**Note:** You can complete the following procedures with an automated coverslipper.

## Complete the run

1. After the run is complete, press the button on the front of the instrument to unload the slides.
2. Remove the covertiles, and place the slides into a TissueTek® Slide Rack.
3. Wash the slides in a staining dish filled with distilled water by moving the rack up and down in the dish several times.
4. If you have not already completed the bluing step, pipette a small amount of the RNAscope® 2.5 LS Bluing Reagent included in the kit onto each slide for **1-2 MIN** followed by rinsing with water or follow your own bluing protocol.

**Note:** Prepare 0.02% (w/v) Ammonia water (bluing reagent) by diluting 28% Ammonia Hydroxide with distilled water.

## Mount the samples

4. Remove the slide rack from the staining dish and dry slides in a **60°C** dry oven for **15 MIN**.

---

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

---

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

---

**IMPORTANT!** Use the EcoMount mounting medium only.

---

7. Carefully place 24 mm x 50 mm coverslip over the tissue section. Avoid trapping air bubbles.
8. Repeat steps 2 and 3 for each slide.
9. Air dry slides for **5 MIN**.

# 7

## Chapter 7. Evaluate the Results

### Evaluate the samples

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 miRNAscope™ 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 miRNAscope™ staining intensity is presented below for a gene with expression level varying between 1 to > 10 copies per cell.

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

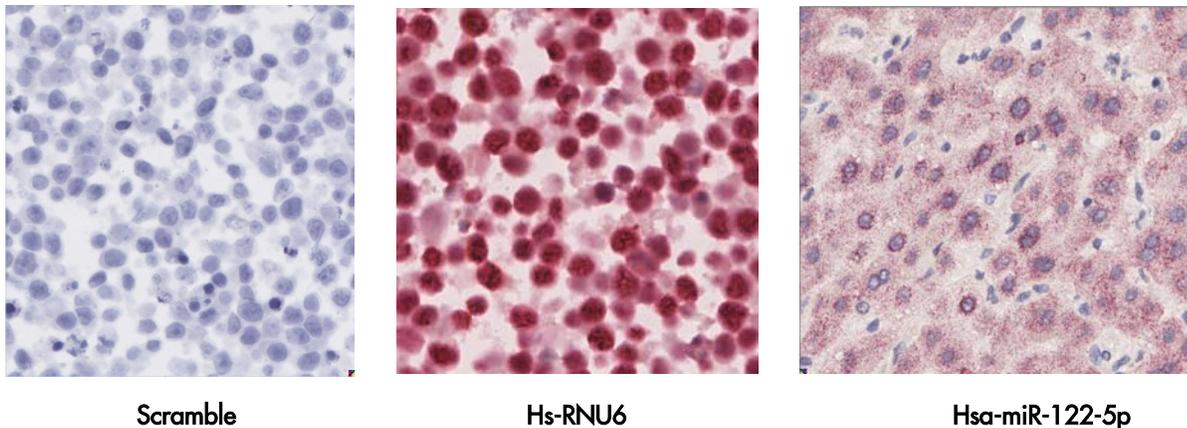
Categorize staining into four grades: 0, 1, 2, and 3 according to the following table:

Staining Score	Microscope Objective Scoring*
0	No staining or less than 1 dot/cell (40X magnification)
1	2–10 dots/cell (visible at 20–40X magnification)
2	11–20 dots/cell and /or <25% dots are in clusters (visible at 20–40X magnification)
3	>20 dots/cell and /or >25% dots are in clusters (visible at 20X magnification)

\* Discount cells with artificially high nuclear background staining.

## Staining examples

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



**Figure 2.** miRNAscope™ LS Assay – Red performed on human cervical cancer sections using the Negative Control Probe - SR-Scramble-S1 (Cat. No. 727888-S1) and Positive Control Probe - SR-RNU6-S1 (Cat. No. 727878-S1), 40X magnification. Detection of Hsa-miR-122-5p in human liver, 40X magnification.

## Troubleshooting

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

- If the signal-to-noise ratio is low due to high background, increase the Epitope Retrieval 2 (ER2) time in increments of five minutes and/or increase the Enzyme time (protease) in increments of ten minutes (see **Appendix A** and **Appendix B** on pages 47 and 50).
- Follow the suggestion above for over-fixed tissues.
- The miRNAscope™ LS Assay uses only the Leica Biosystem BOND Polymer Refine Detection and Bond Polymer Refine Red Detection kits, respectively. Do not use any other chromogen kits.
- Do not shake the contents in the dispensers as this will form bubbles and could 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. The parameters in the staining protocol have been optimized to run the miRNAscope™ Assay on the instrument.

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

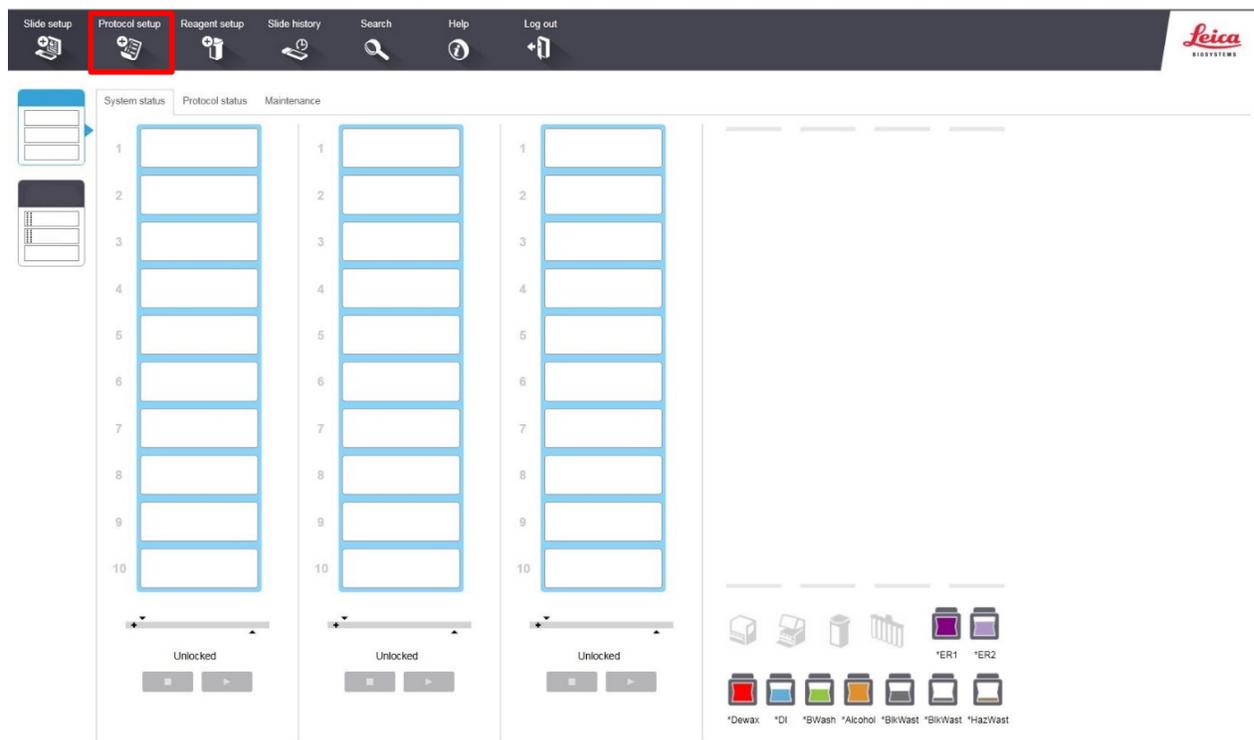
# A

## Appendix A. Edit the Epitope Retrieval Protocol

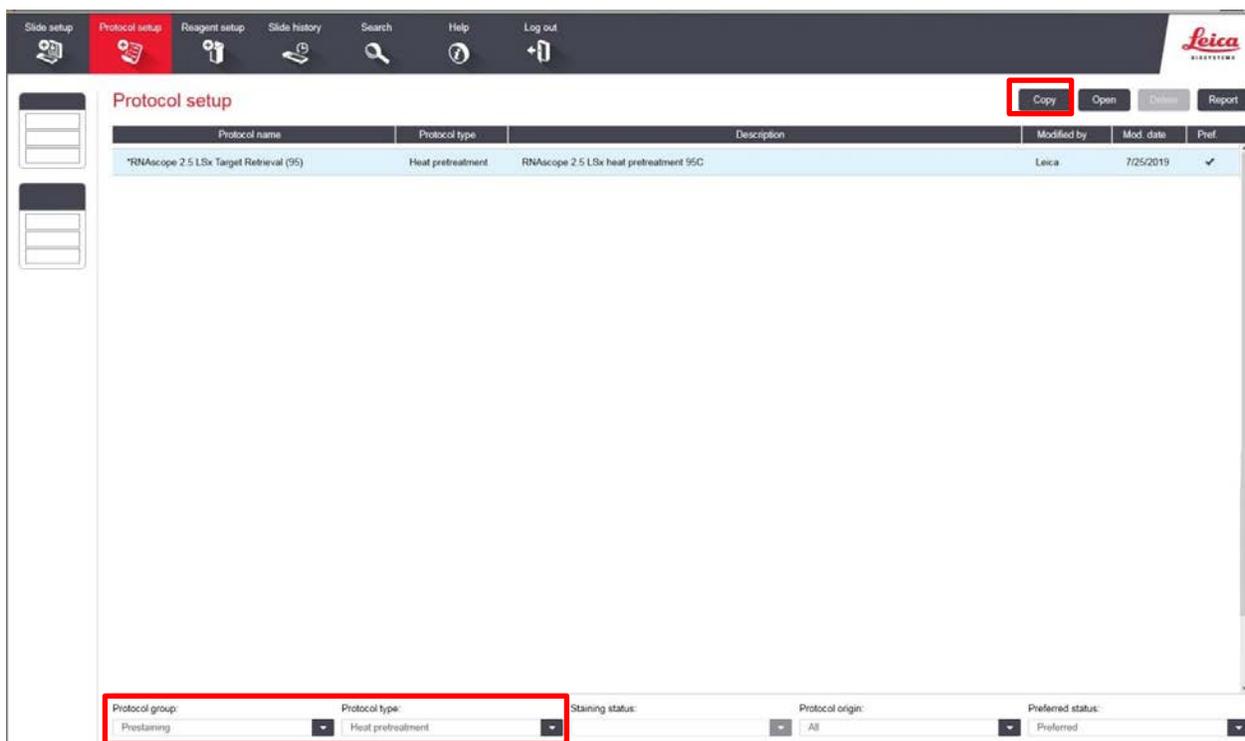
The following example shows how to edit the Epitope Retrieval procedure from within version 6.0 software. You can use similar steps to edit the procedure in version 5.2 software.

### Create a prestaining protocol

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



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



3. Highlight the **\*RNAscope 2.5 LSx Target Retrieval (95)** protocol for version 6.0 software, or highlight the **\*ACD HIER 15 min with ER2 (95)** protocol for version 5.2 software. Select **Copy**.

**Note:** ER2 = Epitope Retrieval 2.

4. Rename the protocol as **RNAscope 2.5 LSx Target Retrieval (88)** (v6.0) or **ACD HIER 15 min with ER2 (88)** (v5.2).
5. Rename the Abbreviated name as **RS2.588** (v6.0) or **ACD88** (v5.2).
6. Rename the Description to **RNAscope 2.5 LSx heat retrieval 88** (v6.0) or **ACD RNAscope heat pretreatment 88** (v5.2).
7. Highlight the third **\*Bond ER Solution 2** step. 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	88°C or 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

Tissue Type	ER2 Incubation Time	Temperature
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
Fresh-frozen tissue*	—	—

\* For fresh-frozen tissue, the HIER step is unnecessary. Select “\*----” for the HIER protocol.

Edit protocol properties

Name: RNAscope 2.5 LSx Target Retrieval (88)

Abbreviated name: RS2.588

Description: RNAscope 2.5 LSx heat retrieval 88C

Preferred

BOND RX Protocol type: Heat pretreatment

Step N°	Wash	Reagent	Supplier	Ambient	Temperature	Inc. (min)	Dispense type
1		*Bond ER Solution 2	Leica Microsystems	✓		0:00	150 µL
2		*Bond ER Solution 2	Leica Microsystems	✓		0:00	150 µL
3		*Bond ER Solution 2	Leica Microsystems		88	15:00	Intermediate
4		*Bond ER Solution 2	Leica Microsystems	✓		0:00	150 µL

Show wash steps

Save Cancel

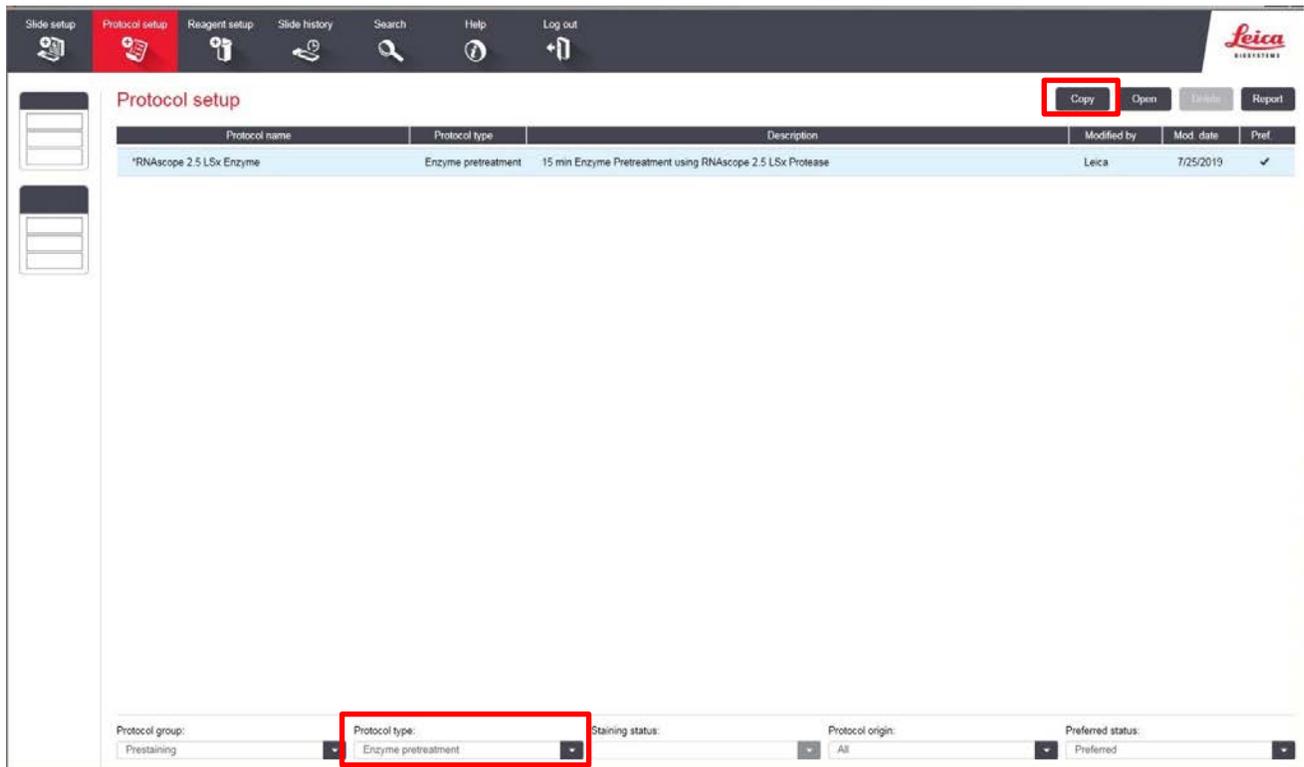
8. Select **Save** to create a protocol for ER2 pretreatment at 88°C.
9. If needed, repeat Steps 1–8 to create a new heating protocol.

# B

## Appendix B. Edit the Protease Protocol

The following example shows how to edit the Protease procedure from within version 6.0 software. You can use similar steps to edit the procedure in version 5.2 software.

1. Select **Enzyme Pretreatment** from the Protocol type menu (bottom left).
2. Highlight the **\*RNAscope 2.5 LSx Enzyme** protocol for version 6.0 software, or highlight the **\*ACD HIER 15 min Protease** protocol for version 5.2 software. Select **Copy**.



3. Rename the protocol to **RNAscope 2.5 LSx 25min** (v6.0) or **ACD 25min Protease** (v5.2).
4. Rename the Abbreviated name to **RS2.5E25** (v6.0) or **25mPro** (v5.2).
5. Rename the Description to **RNAscope 2.5 LSx Protease 25min** (v6.0) or **ACD RNAscope enzyme pretreatment 25min** (v5.2).
6. Highlight the second **\*RNAscope 2.5 LSx Protease** (v6.0) or **\*ACD RNAscope 25min** (v5.2) step. Keep the temperature at **40°C** and set the enzyme incubation time according to the following table:

Tissue Type	Enzyme 1 (Protease III) Incubation Time	Temperature
Brain and spinal cord	15 MIN	40°C
Breast cancer	15 MIN	40°C
Cell pellet	15 MIN	40°C
Colon	15 MIN	40°C
GI tract	15 MIN	40°C
Head and neck cancer	15 MIN	40°C

Tissue Type	Enzyme 1 (Protease III) Incubation Time	Temperature
Heart	15 MIN	40°C
Kidney	15 MIN	40°C
Liver	15 MIN	40°C
Lung	15 MIN	40°C
Lymphoma	15 MIN	40°C
Placenta	15 MIN	40°C
Prostate	15 MIN	40°C
Skin	15 MIN	40°C
Stomach	15 MIN	40°C
Thymus	15 MIN	40°C
Tonsil	15 MIN	40°C
Xenograft	15 MIN	40°C
Fresh-frozen tissue	30 MIN with <b>Protease IV</b>	Ambient

New protocol properties

Name:

Abbreviated name:

Description:

Preferred

BOND RX [Import protocol](#) Protocol type: Enzyme pretreatment

Step N°	Wash	Reagent	Supplier	Ambient	Temperature	Inc. (min)	Dispense type
2		*RNAscope 2.5 LSx Protease	Advanced Cell Diagnostics		40	0:00	150 µL
3		*RNAscope 2.5 LSx Protease	Advanced Cell Diagnostics		40	25:00	50 µL
7		*RNAscope 2.5 LSx H <sub>2</sub> O <sub>2</sub>	Advanced Cell Diagnostics	<input checked="" type="checkbox"/>		10:00	150 µL

Show wash steps

- Select **Save**.
- If needed, repeat Steps 1–7 to create a new protease protocol for different sample types.



# Appendix C. 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) provided before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, visit <http://www.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 [www.cdc.gov/biosafety](http://www.cdc.gov/biosafety)
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030)
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials
- Additional information about biohazard guidelines is available at [www.cdc.gov/](http://www.cdc.gov/)

### 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
- Registration, Evaluation, Authorisation and Restriction of Chemicals (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  
For support, email [support.acd@bio-techne.com](mailto:support.acd@bio-techne.com)  
[www.acdbio.com](http://www.acdbio.com)

Phone 1-510-576-8800

Toll Free 1-877-576-3636

