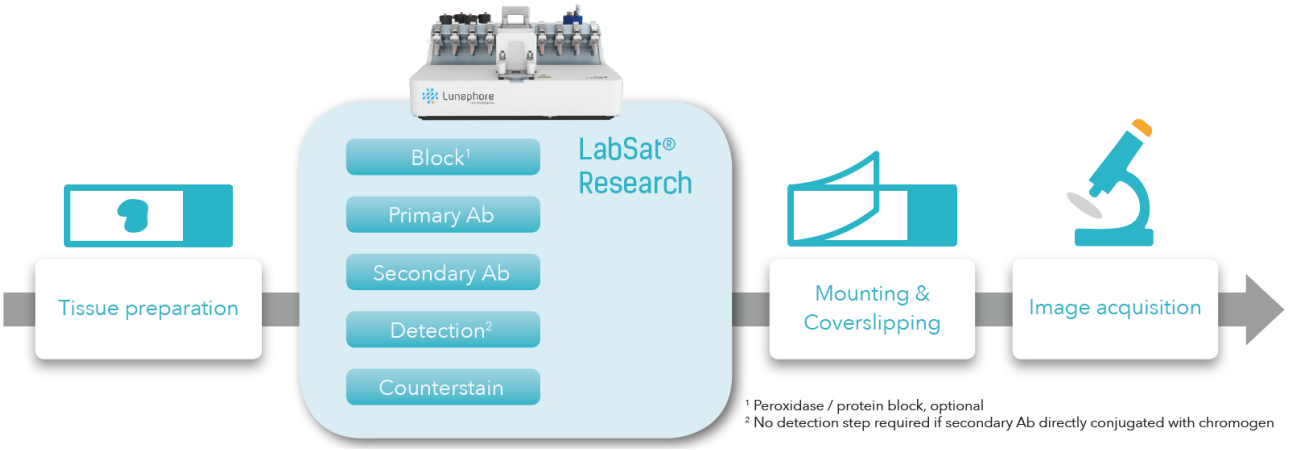
Short Guide: FS IHC staining LabSat®

This short guide will help you get started with protocols for FS IHC stainings on LabSat®. LabSat® handles the staining steps from blocking steps to counterstaining and without any user intervention.
LabSat® is CE/UKCA/UL marked and is For Research Use Only. Not for use in diagnostic procedures.



Material needed

	Description	Brand	Catalog number
Equipment	LabSat®	Lunaphore	LS01
	Microfluidic Chips (Microfluidic Kit, LabSat® Distribution Chips)	Lunaphore	MK01, MK02
	Scanner/microscope	N/A	N/A
	Image analysis software	N/A	N/A
	Hot plate capable to reach 45 °C	N/A	N/A
	Orbital shaker capable of speed of 65 rpm	N/A	N/A
Staining reagents[1]	Primary antibodies	N/A	N/A
	ImmPRESS® HRP Horse Anti-Mouse IgG Polymer Detection Kit, Peroxidase	Vector Laboratories	MP-7402
	ImmPACT® DAB Substrate, Peroxidase (HRP)	Vector Laboratories	SK-4105
	BLOXALL® Endogenous Blocking Solution	Vector Laboratories	SP-6000
	Hematoxylin Counterstain	Vector Laboratories	H-3401
	Staining Buffer[2]	Lunaphore	BU01
	Ethanol 70% (diluted in DIW, for automated in-protocol washes)	Fischer Chemicals	E/0650DF/15
Pre- & post- processing reagents[3]	NBF™	Thermo Scientific	5701
	Acetone	N/A	N/A
	Ethanol 100% (will be used at several concentrations)	Fischer Chemicals	E/0650DF/15
	Tap Water	N/A	N/A
	Staining Buffer[2]	Lunaphore	BU01
	IHC Mounting medium, preferably DPX™	N/A	N/A
	Deionized or demineralized water (DIW)	N/A	N/A

[1] The system is open and can be used with various detection systems.
[2] Use of Lunaphore buffers (BU01) is recommended, but other buffer solutions can be used.
[3] The reagents listed are required to perform the pre-processing procedure for FS slides recommended by Lunaphore. Other reagents and methods can be used as long as the slide is fixed before being processed by LabSat®.

Lunaphore standard pre-processing IHC procedure for FS slides

For long term FS storage (-80°): Lunaphore recommends the neutral buffered formaline (NBF) fixation protocol described below. Acetone fixation is recommended if good results are not obtained with NBF fixation (especially for CD45 LCA marker).

NBF fixation	Acetone fixation
• Place the samples on a hot plate at 45°C for 1 minute or let it thaw out 15 minutes at room temperature. • Incubate for 3 minutes in neutral buffered formalin (NBF) 10% on an orbital shaker and under the hood. • Wash in DIW, 1 minute. • Store samples in washing buffer until loading into LabSat®.	• Place the samples on a hot plate at 45°C for 1 minute under the hood. • Incubate for 3 minutes in acetone on an orbital shaker and under the hood. • Bake samples for 1 min at 45°C under the hood. • Store samples in washing buffer until loading into LabSat®.

For fresh samples: Lunaphore recommends the NBF or acetone fixation described below.

NBF or acetone fixation
• Incubate for 3 minutes in neutral buffered formalin (NBF) 10% or acetone, on an orbital shaker and under the hood. • Wash in DIW, 1 minute. • Store samples in washing buffer until loading into LabSat®.

Protocol creation and loading

1. If necessary, create new reagents in the REAGENTS tab.
2. From the PROTOCOLS tab, one can load an existing protocol directly by clicking the **Add to queue** button and go to step 4. Alternatively, a new one can be created by clicking the **Add new** button and following the instructions in step 3 below.
3. Protocol creation
    a. Select the FS > IHC (Chromogenic). Click OK to create the template.
    b. Edit name of the protocol if needed (it is "FS Chromogenic" by default).
    c. If needed, complete the **REAGENT KIT** and **DESCRIPTION** fields that are below the estimated **TOTAL TIME**.
    d. Select the Washing buffer from the drop-down list.
    e. Configure the protocol by selecting reagents and incubation times by expanding each step with the button. You can also add or remove steps using the buttons or change their order via drag and drop.
        i. If endogenous activity is observed/known in this tissue sample, activate the Peroxidase block step.
        ii. If non-specific binding of the secondary antibodies is observed/known, add the Protein block step.
        Tip: Dilute the primary antibody in Protein block. This allows time reduction by performing both steps at once.
    f. Status dots are displayed for each step and whole protocol. If all of them are green, no obvious problem has been detected. Hovering the mouse over an orange dot (warning) or red dot (blocking) reveals the error.
    g. Click the **Save** button to save your protocol.
4. Full details of the protocol can be displayed by clicking the button. List of required reagents and their required volumes (without priming) are displayed, and priming volumes are indicated in brackets.

Reagent preparation

Prepare an adequate volume of reagents:

Volume to prepare = Priming Volume + (Dispense volume x n)

Where **n** is the number of slides to be stained.

- Priming volume is 120 µl for small reservoirs and 500 µl for large reservoirs.
- If using the BOOST option (available for the primary and secondary antibody step), the Dispense volume is increased to 280 µL (instead of 180 µL).

The reservoir attribution should be:

- Blocking reagents, primary and secondary antibodies, substrate, and counterstain are loaded in small reservoirs.
- DIW, washing buffer, and alcohol are loaded in large reservoirs.

Large reservoirs should always be loaded with at least 10 mL. Even if less is required, prepare more of a given reagent/buffer and reuse it for your next protocols.

Therefore, Lunaphore recommends the following configuration:

		Description	Dilution and diluent	Volume to prepare
Large reservoirs	A	Staining Buffer	1:10 with DIW	50 mL
	B	DIW	RTU	
	D	Ethanol	70% in DIW	

		Description	Dilution and diluent	Volume to prepare			
				For n stainings		n=1	n=2
Small reservoirs	1	Peroxidase block	RTU[2]	120 µL + (180 µL x n)		300 µL	480 µL
	2	Protein block	RTU[2]				
	3-5	Primary antibodies	N/A	120 µL + (180 µL x n) or 120 µL + (280 µL x n)[1]		300 µL or 400 µL[1]	480 µL or 680 µL[1]
	6	Secondary antibodies	N/A				
	7	DAB substrate	3% DAB reagent, diluted in DAB diluent[2]	DAB reagent	3.6 µL + (10.8 µL x n)	14.4 µL DAB reagent + 465.6 µL DAB diluent	25.2 µL DAB reagent + 814.8 µL DAB diluent
				DAB diluent	116.4 µL + (349.2 µL x n)		
	8	Hematoxylin	RTU[2]	120 µL + (180 µL x n)		300 µL	480 µL

[1] If BOOST option is selected.

[2] If using Vector Laboratories products.

For detailed information about optimized protocols for specific markers, see Optimized Markers Brochure – FS.