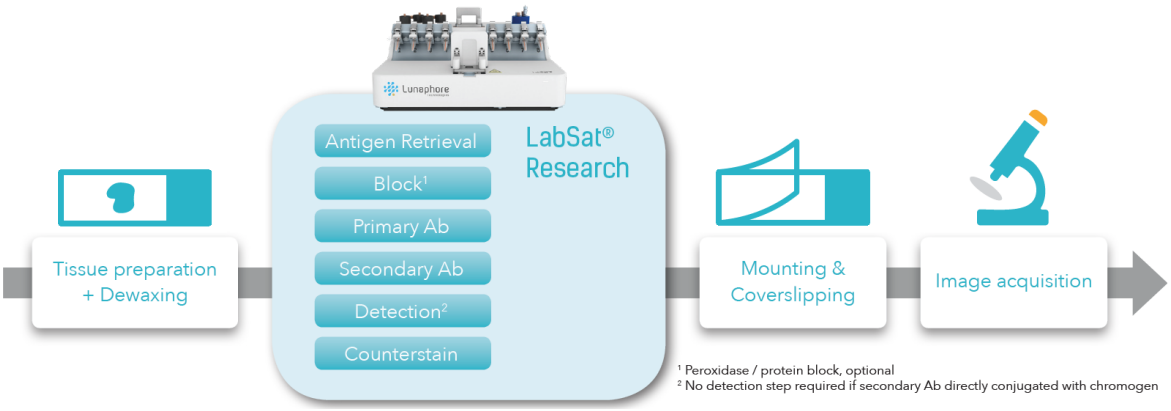
Short Guide: FFPE IHC staining on LabSat®

This short guide will help you get started with protocols for FFPE IHC stainings on LabSat®. LabSat® handles the staining steps from antigen retrieval 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
	Oven capable of 65°C incubation	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
	Antigen Retrieval Solution pH 6[2]	Lunaphore	BU04
	Antigen Retrieval Solution pH 9[2]	Lunaphore	BU05
Pre- & post-processing reagents[3]	Histoclear™ (Xylene substitute)	National Diagnostics	HS-200
	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, BU04 and BU05) is recommended, but other buffer solutions can be used.
[3] The reagents listed are required to perform the pre-processing procedure for FFPE slides recommended by Lunaphore. Other reagents and methods can be used to deparaffinize and re-hydrate the slide before processing on LabSat®.

Lunaphore standard pre-processing IHC procedure for FFPE slides

- Bake samples for 10 minutes at 65°C in an oven,
- Dewax samples for 10 minutes in Histoclear™ solution on a shaker and under the hood.
- Perform the following sequential washing steps in separate containers:
    - Histoclear™, 30 seconds
    - Ethanol 100%, 30 seconds
    - Ethanol 100%, 10 seconds
    - Ethanol 95%, 10 seconds
    - Ethanol 70%, 10 seconds
    - Ethanol 40%, 10 seconds
    - Tap water, 30 seconds
    - Washing buffer (Staining Buffer or TBS) until staining

Protocol creation and loading

1. If necessary, create new reagents in the REAGENTS tab.
2. From the PROTOCOLS tab, load an existing protocol directly by clicking the Add to queue button and go to step 4. Alternatively, create a new one by clicking the Add new button and follow the instructions in step 3 below.
3. Protocol creation
    a. Select the FFPE > IHC (Chromogenic). Click OK to create the template.
    b. Edit name of the protocol if needed (it is “FFPE 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, antigen retrieval, 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	
	C	Antigen Retrieval Solution pH 6 or 9	1:10 in DIW	If n < 4, prepare at least 5 mL
				If n > 4, prepare 500 µL + (1100 µL x n)
	D	Ethanol	70% in DIW	50 mL

		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	RTU[2]			
	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 – FFPE.