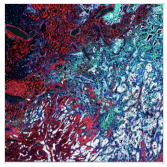

Version 1

Aug 10, 2020

FFPE Tissue Pre-treatment Before t-CyCIF on Leica Bond RX V.1

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1 Works for me dx.doi.org/10.17504/protocols.io.4zpgx5n

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ABSTRACT

Tissue-based cyclic immunofluorescence (t-CyCIF) is optimized for FFPE specimens mounted on glass slides. Dewaxing and antigen retrieval are important steps to remove wax and expose antigenic sites. This protocol describes dewaxing and antigen retrieval on a Leica Bond RX automated slide processor; similar instruments are manufactured by Ventana or Dako and are commonly found in histopathology core facilities. t-CyCIF can also be performed following manual de-waxing and antigen retrieval (e.g. microwaving slides in citrate buffer or using a pressure cooker).

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25359

MATERIALS

NAME	CATALOG #	VENDOR
Odyssey® Blocking Buffer (PBS)	927-40000 927-40100	LI-COR
200 proof ethanol		
Dewax Solution	AR9222	Leica Biosystems
Epitope Retrieval Solution 2 BOND	AR9640	Leica Biosystems
Hoechst 33342	4082	Cell Signaling Technology
20X Phosphate Buffered Saline	28348	Thermo Fisher Scientific
Bond Universal Covertiles	S21.4611	Leica Biosystems

MATERIALS TEXT

Fluorescently-conjugated secondary antibodies (experiment-specific)

EQUIPMENT

NAME	CATALOG #	VENDOR
Leica BOND RX	3342171	

1 Create a protocol file named "t-CyCIF" on the Leica Bond RX (see user manual for instrument-specific details):

- **CRITICAL STEP** We recommend creating a protocol file named “t-CyCIF” in the Leica Bond RX with the following conditions and saving for future use.

Leica BOND RX 3342171
https://www.leicabiosystems.com/ihc-ish-fish/ihc-ish-instruments/products/leica-bond-rx/

1.1 Bake FFPE slides at **60 °C** for **00:30:00** .

1.2 Dewax by rinsing three times with **150 µl** preheated (**60 °C**) Bond dewax solution, following by 3 rinses of **150 µl** , 200 proof ethanol.

1.3 Remove the Bond dewax solution. Add **150 µl** Bond ER1 solution for antigen retrieval and incubate at **99 °C** for **00:20:00** .

1.4 Remove the Bond ER1 solution and block for **00:30:00** with **150 µl** Odyssey® blocking buffer at **Room temperature** .

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1.5 Remove the blocking buffer and incubate with **150 µl** secondary antibody solution by incubating for **01:00:00** at **Room temperature** .

1.6 Remove the secondary antibody solution and incubate with **150 µl** Hoechst solution for **00:30:00** at **Room temperature** .

2 Prepare reagent chambers for Leica Bond RX.

- **CRITICAL STEP** Prior to the first cycle of t-CyCIF, a prestaining (blocking) step is performed by incubating tissues with a mixture of appropriate secondary antibodies to block non-specific binding sites in the tissue. Secondary antibodies are chosen based on the species and isotypes of the unconjugated antibodies used in the first t-CyCIF cycle.
- **CRITICAL STEP** Avoid using Alexa Fluor 546-, Alexa Fluor 568- or Alexa Fluor 594-conjugated secondary antibodies, as these fluorophores are resistant to bleaching.

2.1 Fill chamber 1 with **30 mL** of 1X PBS.

2.2 Fill chamber 2 with **7 mL** of Odyssey® blocking buffer.

2.3 Fill chamber 3 with **2 mL** of the appropriate secondary antibodies conjugated with Alexa Fluor–488, Alexa Fluor–555, or Alexa Fluor–647 diluted in Odyssey® blocking buffer (1:1000, vol:vol).

2.4 Open the lids of the chambers and put them in the chamber tray in the Leica Bond RX.

3 Place chambers in reagent tray and insert into Leica Bond RX.

4 Create a new study on the Leica Bond RX, select “t-CyCIF” file created as the protocol, add each slide to the study, and print labels for each FFPE slide. The Bond requires barcoded labels on all slides to process them.

- **CRITICAL STEP** Center the slide stickers evenly so that the Bond RX can scan the barcodes on the stickers correctly.

5 Place all labeled slides onto a slide tray, cover the slides with Bond Universal Covertiles, and insert the slide tray into the machine.

- **CRITICAL STEP** Be sure to place the Covertiles right-side-up, using the “Leica” etched on each Covertile as an orientation guide.

6 The machine will scan the barcodes on the stickers of the slides and reagent chambers. Check the label readings by

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hovering the mouse over the slide labels. If all of the slides have been recognized correctly, then click the START button to run the protocol. This will take approximately **04:00:00** .

7 Remove slides from the Leica Bond RX and place into 1X PBS.

■ **PAUSE POINT** Slides can be stored in 1X PBS at **4 °C** for several days after processing on the Bond RX. Ensure that the entire tissue is covered in 1X PBS; otherwise the tissue will dry out and yield poor results.

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