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).

EXTERNAL LINK
www.cycif.org

DOI
dx.doi.org/10.17504/protocols.io.bji2kkge

PROTOCOL CITATION
Jia Ren Lin, Benjamin Izar, Zoltan Maliga, Yu-An Chen, Giorgio Gaglia, Ziming Du, Clarence Yapp, Shaolin Mei, Sandro Santagata, Peter Sorger 2020. FFPE Tissue Pre-treatment Before t-CyCIF on Leica Bond RX. protocols.io
https://dx.doi.org/10.17504/protocols.io.bji2kkge

LICENSE
This is an open access protocol distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

CREATED
Aug 10, 2020

LAST MODIFIED
Aug 10, 2020
PROTOCOL INTEGER ID
40250

MATERIALS TEXT
MATERIALS

200 proof ethanol Contributed by users
Epitope Retrieval Solution 1 BOND Leica
Biosystems Catalog #AR9961
Dewax Solution Leica
Biosystems Catalog #AR9222
Hoechst 33342 Cell Signaling
Technology Catalog #4082
20X Phosphate Buffered Saline Thermo Fisher
Scientific Catalog #28348
Bond Universal Covertiles Leica
Biosystems Catalog #S21.4611
Intercept (PBS) Blocking Buffer LI-
COR Catalog #927-70001

Fluorescently-conjugated secondary antibodies (experiment-specific)

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 \(150 \mu l\) Intercept® (PBS) Blocking Buffer at \(\text{Room temperature}\).

1.5 Remove the blocking buffer and incubate with \(150 \mu l\) secondary antibody solution by incubating for \(01:00:00\) at \(\text{Room temperature}\).

1.6 Remove the secondary antibody solution and incubate with \(150 \mu l\) Hoechst solution for \(00:30:00\) at \(\text{Room temperature}\).

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 Intercept® (PBS) 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 Intercept® (PBS) 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 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 them 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.