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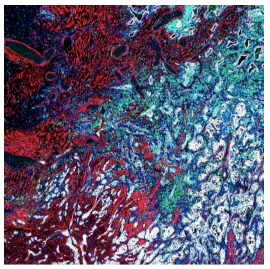
FFPE Tissue Pre-treatment Before t-CyCIF on Leica Bond RX V.2



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Jia Ren Lin[1], Benjamin Izar[1,2], Zoltan Maliga[1,2], Yu-An Chen[1], Giorgio Gaglia[1,2,3], Ziming Du[1,2], Clarence Yapp[1], Shaolin Mei[1], Sandro Santagata[1,2,3], Peter Sorger[1,2]

[1]Laboratory of Systems Pharmacology, Harvard Medical School, Boston, MA;
[2]Ludwig Center for Cancer Research at Harvard, Harvard Medical School, Boston, MA;
[3]Department of Pathology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA

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Madison Tyler
Laboratory of Systems Pharmacology

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We use this protocol and it's working

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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).

Materials

MATERIALS

- 200 proof ethanol
- 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.

Equipment

new equipment	NAME
Leica BOND RX	BRAND
3342171	SKU
https://www.leicabiosystems.com/ihc-ish-fish/ihc-ish-instruments/products/leica-bond-rx/	SPECIFICATIONS

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 Intercept® (PBS) Blocking Buffer at Room temperature .

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 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.