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6-plex IF Protocol on the BOND RX (Leica Biosystems)

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ABSTRACT

A 6-plex immunofluorescence protocol was optimized on Leica Bond RX Autostainer.

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Preparation

1 **Multiplex IF assay on the Bond RX Autostainer (Leica Biosystems) using the Opal multiplex IHC system (PerkinElmer/Akoya Biosciences Cat# NEL871001KT) and**

the BOND Polymer Refine Detection Kit (Leica Biosystems Cat# DS9800).

- Prepare the antibody and the fluorophore dilutions in 6mL Leica BOND tubes (see Ficial M et al for details about reagents, dilutions and order of antibodies).

- Fill an empty 7ml Leica BOND container with DAPI.

- Fill an empty 30ml Leica BOND container with Dako Protein Block.

2 Bake the unstained FFPE slides in the Isotemp Oven for 30 mins at 60°C.

Leica BOND RX Protocol

3 Deparaffinization and Rehydration

- BOND Dewax Solution (Leica Biosystems Cat# AR9222)
Time. (min): 30:00, Temperature: 72°C, Dispense type: open.

- Rehydration.

4 Bond ER Solution 1 (Leica Biosystems Cat# AR9961; citrate, pH 6.0)
Time. (min): 10:00, Temperature: 98°C, Dispense type: 150 µL.

5 Deionized Water
Time. (min): 2:00; Temperature: Ambient; Dispense type: 150 µL.

6 Bond Wash Solution (Leica Biosystems Cat# AR9590)
Time. (min): 2:00; Temperature: Ambient; Dispense type: 150 µL.

7 Peroxide Block (Leica Biosystems Cat# DS9800)
Time. (min): 10:00; Temperature: Ambient; Dispense type: 150 µL.

8 Bond Wash Solution (Leica Biosystems Cat# AR9590)
Time. (min): 4:00; Temperature: Ambient; Dispense type: 150 µL.

9 Dako Protein Block (Agilent Cat# X090930-2)

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Time. (min): 15:00; Temperature: Ambient; Dispense type: 150 µL.

10 Primary Antibody (diluted in either Leica Biosystems BOND Primary Antibody Diluent Cat# AR9352 or Biocare Medical DaVinci Green Diluent Cat# PD900 M)
Time. (min): 40:00; Temperature: Ambient; Dispense type: 150 µL.

11 Bond Wash Solution (Leica Biosystems Cat# AR9590)
Time. (min): 4:00; Temperature: Ambient; Dispense type: 150 µL.

12
Post-Primary (Leica Biosystems Cat# DS9800) *
Time. (min): 15:00; Temperature: Ambient; Dispense type: 150 µL.

*Post-Primary should be used after mouse anti-human primary antibodies only; when using rabbit anti-human primary antibodies, please go directly to step 14.

For TIM-3 this step was replaced by:
Rabbit anti Goat Link
Time. (min): 15:00; Temperature: Ambient; Dispense type: 150 µL

13 Bond Wash Solution (Leica Biosystems Cat# AR9590)
Time. (min): 4:00; Temperature: Ambient; Dispense type: 150 µL.

14 Polymer (Leica Biosystems Cat# DS9800)
Time. (min): 10:00; Temperature: Ambient; Dispense type: 150 µL.

15 Bond Wash Solution (Leica Biosystems Cat# AR9590)
Time. (min): 6:00; Temperature: Ambient; Dispense type: 150 µL.

16 Opal Fluorophore (PerkinElmer/Akoya Biosciences Cat# NEL871001KT)
Time. (min): 5:00; Temperature: Ambient; Dispense type: 150 µL.

17 Bond Wash Solution (Leica Biosystems Cat# AR9590)
Time. (min): 6:00; Temperature: Ambient; Dispense type: 150 µL.

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18 Bond ER Solution 1(Leica Biosystems Cat# AR9961; citrate, pH 6.0)
Time. (min): 10:00, Temperature: 98°C, Dispense type: 150 µL.

19 **Start a new cycle of staining from step 5 OR go to step 20**

20 Last cycle of staining:

20.1 Deionized Water
Time. (min): 2:00; Temperature: Ambient; Dispense type: 150 µL.

20.2 DAPI (PerkinElmer/Akoya Biosciences Cat# NEL871001KT)
Time. (min): 10:00; Temperature: Ambient; Dispense type: 150 µL.

20.3 Manually coverslip using Invitrogen ProLong Diamond Antifade Mountant (Thermo Fisher Scientific Cat# P36962).

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