

## Leica Bond Platform Protocol Summary

### IHC Reagents (Leica Platform):

- Leica Bond Wash Solution (Leica, Cat# AR9590)
- Bond Polymer Refine Detection Kit (Leica, Cat# DS9800)
- Bond Epitope Retrieval Solution 2 (Leica, Cat# AR9640)
- Envision FLEX Antibody Diluent (Dako, Cat# S0809)

### Pre-treatment: \*HIER 20min with ER2

### Bond RX Protocol: \*H2(20)

1	*Bond ER Solution 2 Step Reagent Supplier: Leica Microsystems	Step type: Reagent Inc. (min): 0:00 Temperature: Ambient Dispense type: 150 µL
2	*Bond ER Solution 2 Step Reagent Supplier: Leica Microsystems	Step type: Reagent Inc. (min): 0:00 Temperature: Ambient Dispense type: 150 µL
3	*Bond ER Solution 2 Step Reagent Supplier: Leica Microsystems	Step type: Reagent Inc. (min): 20:00 Temperature: 100 Dispense type: Intermediate
4	*Bond ER Solution 2 Step Reagent Supplier: Leica Microsystems	Step type: Reagent Inc. (min): 0:00 Temperature: Ambient Dispense type: 150 µL
5	*Bond Wash Solution Step Reagent Supplier: Leica Microsystems	Step type: Wash Inc. (min): 0:00 Temperature: Ambient Dispense type: 150 µL
6	*Bond Wash Solution Step Reagent Supplier: Leica Microsystems	Step type: Wash Inc. (min): 0:00 Temperature: Ambient Dispense type: 150 µL
7	*Bond Wash Solution Step Reagent Supplier: Leica Microsystems	Step type: Wash Inc. (min): 0:00 Temperature: Ambient Dispense type: 150 µL
8	*Bond Wash Solution Step Reagent Supplier: Leica Microsystems	Step type: Wash Inc. (min): 0:00 Temperature: Ambient Dispense type: 150 µL
9	*Bond Wash Solution Step Reagent Supplier: Leica Microsystems	Step type: Wash Inc. (min): 3:00 Temperature: Ambient

	Dispense type: 150 µL
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**Open Wash IHC ProcF 30 Min inc**  
**Bond RX Protocol: \* oIHCF30'**

1	*Peroxide Block Step Reagent Supplier: Leica Microsystems	Step type: Reagent Inc. (min): 5:00 Temperature: Ambient Dispense type: 150 µL
2	*Bond Wash Solution Step Reagent Supplier: Leica Microsystems	Step type: Wash Inc. (min): 0:00 Temperature: Ambient Dispense type: 150 µL
3	*Bond Wash Solution Step Reagent Supplier: Leica Microsystems	Step type: Wash Inc. (min): 0:00 Temperature: Ambient Dispense type: Open
4	*Bond Wash Solution Step Reagent Supplier: Leica Microsystems	Step type: Wash Inc. (min): 0:00 Temperature: Ambient Dispense type: 150 µL
5	Primary Step Reagent Supplier:	Step type: Reagent Inc. (min): 30:00 Temperature: Ambient Dispense type: 150 µL
6	*Bond Wash Solution Step Reagent Supplier: Leica Microsystems	Step type: Wash Inc. (min): 0:00 Temperature: Ambient Dispense type: 150 µL
7	*Bond Wash Solution Step Reagent Supplier: Leica Microsystems	Step type: Wash Inc. (min): 0:00 Temperature: Ambient Dispense type: 150 µL
8	*Bond Wash Solution Step Reagent Supplier: Leica Microsystems	Step type: Wash Inc. (min): 0:00 Temperature: Ambient Dispense type: 150 µL
9	*Post Primary Step Reagent Supplier: Leica Microsystems	Step type: Reagent Inc. (min): 8:00 Temperature: Ambient Dispense type: 150 µL
10	*Bond Wash Solution Step Reagent Supplier: Leica Microsystems	Step type: Wash Inc. (min): 2:00 Temperature: Ambient Dispense type: 150 µL

11	*Bond Wash Solution Step Reagent Supplier: Leica Microsystems	Step type: Wash Inc. (min): 2:00 Temperature: Ambient Dispense type: 150 µL
12	*Bond Wash Solution Step Reagent Supplier: Leica Microsystems	Step type: Wash Inc. (min): 2:00 Temperature: Ambient Dispense type: 150 µL
13	*Polymer Step Reagent Supplier: Leica Microsystems	Step type: Reagent Inc. (min): 8:00 Temperature: Ambient Dispense type: 150 µL
14	*Bond Wash Solution Step Reagent Supplier: Leica Microsystems	Step type: Wash Inc. (min): 2:00 Temperature: Ambient Dispense type: 150 µL
15	*Bond Wash Solution Step Reagent Supplier: Leica Microsystems	Step type: Wash Inc. (min): 2:00 Temperature: Ambient Dispense type: Open
16	*Bond Wash Solution Step Reagent Supplier: Leica Microsystems	Step type: Wash Inc. (min): 2:00 Temperature: Ambient Dispense type: 150 µL
17	*Deionized Water Step Reagent Supplier:	Step type: Wash Inc. (min): 0:00 Temperature: Ambient Dispense type: 150 µL
18	*Deionized Water Step Reagent Supplier:	Step type: Wash Inc. (min): 0:00 Temperature: Ambient Dispense type: 150 µL
19	*Mixed DAB Refine Step Reagent Supplier: Leica Microsystems	Step type: Reagent Inc. (min): 0:00 Temperature: Ambient Dispense type: 150 µL
20	*Mixed DAB Refine Step Reagent Supplier: Leica Microsystems	Step type: Reagent Inc. (min): 10:00 Temperature: Ambient Dispense type: 150 µL
21	*Deionized Water Step Reagent Supplier:	Step type: Wash Inc. (min): 0:00 Temperature: Ambient Dispense type: 150 µL

22	*Deionized Water Step Reagent Supplier:	Step type: Wash Inc. (min): 0:00 Temperature: Ambient Dispense type: 150 µL
23	*Deionized Water Step Reagent Supplier:	Step type: Wash Inc. (min): 0:00 Temperature: Ambient Dispense type: Open
24	*Deionized Water Step Reagent Supplier:	Step type: Wash Inc. (min): 0:00 Temperature: Ambient Dispense type: 150 µL
25	*Hematoxylin Step Reagent Supplier: Leica Microsystems	Step type: Reagent Inc. (min): 5:00 Temperature: Ambient Dispense type: 150 µL
26	*Deionized Water Step Reagent Supplier:	Step type: Wash Inc. (min): 0:00 Temperature: Ambient Dispense type: 150 µL
27	*Bond Wash Solution Step Reagent Supplier: Leica Microsystems	Step type: Wash Inc. (min): 2:00 Temperature: Ambient Dispense type: 150 µL
28	*Deionized Water Step Reagent Supplier:	Step type: Wash Inc. (min): 0:00 Temperature: Ambient Dispense type: 150 µL