



6197 Cornerstone Court E, Ste 102
San Diego, CA 92121

UltraPlex mxIF **Multiplex Immunofluorescence Staining Protocol – suggested for BOND RX**

For use with ***UltraPlex mxIF*** “A” or “B” panels. “A” panels are labeled with 490, 550, 650 and 750 nm fluors. Please ensure that your imaging scanner or fluorescent microscope can detect these wavelengths. Alternatively, you may want to use a “B” kit labeled with 490, 550, 594 and 650 nm fluors. Please contact us if you have any questions about which kits to select. “B” panels are commonly used with spectral imaging scanners.

Not recommended for use with UltraPlex panels HI-1A and HI-1B.

“A & B” panels are optimized for use on FFPE tissue sections.

Materials Provided – Store all components at 2-8°C

1. Vial 1: Antibody Diluent Solution (see notes for composition)
2. Vial 2: Protein Block Solution (see notes for composition)
3. Vials 3-6: Individual Primary Antibody-Hapten Conjugates (suggested dilution for use is 1/100)
4. Vials 7-10: Individual Secondary Anti-Hapten Fluor Conjugates (suggested dilution for use is 1/100)

Materials Not Provided

Suggested mounting medium, Fluoroshield plus DAPI (ImmunoBioSciences, Inc, Cat # AR-6501-01)

All Bond Rx Components

SUGGESTED BOND RX PROTOCOL

BOND RX dewax protocol *D

Step Reagent

1 *Bond Dewax Solution

Supplier: Leica Microsystems

Step type: Reagent Inc. (min): 0:30 Temperature: 72 Dispense type: 150 µL

Step Reagent

2 *Bond Dewax Solution

Supplier: Leica Microsystems

Step type: Reagent Inc. (min): 0:00 Temperature: 72 Dispense type: 150 µL

Step Reagent

3 *Bond Dewax Solution

Supplier: Leica Microsystems

Step type: Reagent Inc. (min): 0:00 Temperature: Ambient Dispense type: 150 µL



Step Reagent

4 *Alcohol

Supplier: Not applicable

Step type: Reagent Inc. (min): 0:00 Temperature: Ambient Dispense type: 150 µL

Step Reagent

5 *Alcohol

Supplier: Not applicable

Step type: Wash Inc. (min): 0:00 Temperature: Ambient Dispense type: 150 µL

Step Reagent

6 *Alcohol

Supplier: Not applicable

Step type: Wash Inc. (min): 0:00 Temperature: Ambient Dispense type: 150 µL

Step Reagent

7 *Bond Wash Solution

Supplier: Leica Microsystems

Step type: Wash Inc. (min): 0:00 Temperature: Ambient Dispense type: 150 µL

Step Reagent

8 *Bond Wash Solution

Supplier: Leica Microsystems

Step type: Wash Inc. (min): 0:00 Temperature: Ambient Dispense type: 150 µL

Step Reagent

9 *Bond Wash Solution

Supplier: Leica Microsystems

Step type: Wash Inc. (min): 0:00 Temperature: Ambient Dispense type: 150 µL

Pre-treatment Antigen Retrieval: *HIER 20 minutes with ER2

Step Reagent

1 *Bond ER Solution 2

Supplier: Leica Microsystems

Step type: Reagent Inc. (min): 0:00 Temperature: Ambient Dispense type: 150 µL

Step Reagent

2 *Bond ER Solution 2

Supplier: Leica Microsystems

Step type: Reagent Inc. (min): 0:00 Temperature: Ambient Dispense type: 150 µL

Step Reagent

3 *Bond ER Solution 2

Supplier: Leica Microsystems

Step type: Reagent Inc. (min): 20:00 Temperature: 100 Dispense type: Intermediate

Step Reagent

4 *Bond ER Solution 2

Supplier: Leica Microsystems

Step type: Reagent Inc. (min): 0:00 Temperature: Ambient Dispense type: 150 µL

Step Reagent

5 *Bond Wash Solution



Supplier: Leica Microsystems

Step type: Wash Inc. (min): 0:00 Temperature: Ambient Dispense type: 150 µL

Step Reagent

6 *Bond Wash Solution

Supplier: Leica Microsystems

Step type: Wash Inc. (min): 0:00 Temperature: Ambient Dispense type: 150 µL

Step Reagent

7 *Bond Wash Solution

Supplier: Leica Microsystems

Step type: Wash Inc. (min): 0:00 Temperature: Ambient Dispense type: 150 µL

Step Reagent

8 *Bond Wash Solution

Supplier: Leica Microsystems

Step type: Wash Inc. (min): 0:00 Temperature: Ambient Dispense type: 150 µL

Step Reagent

9 *Bond Wash Solution

Supplier: Leica Microsystems

Step type: Wash Inc. (min): 3:00 Temperature: Ambient Dispense type: 150 µL

Custom Staining Protocol:

- 1) Dilute antibodies in appropriate amount of Cell IDx antibody diluent. Suggested dilution is 1/100 dilution of concentrate. For example, to make 200 µl of primary antibody solution add 2 ul of each primary x 4 antibodies = 8 µl total primary Abs + 192 µl Antibody Diluent. If individual stains are required, dilute 2 µl of primary antibody with 198 µl Antibody Diluent. For example, to make 200 µl of secondary antibody solution add 2 µl of each secondary x 4 antibodies = 8 µl total primary Abs + 192 µl Antibody Diluent. If individual stains are required, dilute 2 µl of secondary antibody with 198 µl Antibody Diluent. Make sure to prepare enough antibody solution to account for the dead volume on the autostainer.

Step Reagent

1. Water

Supplier: *Not applicable*

Step type: Reagent Inc. (min): 0:00 Temperature: Ambient Dispense type: 150 µL

Step Reagent

2. *Bond Wash Solution

Supplier: *Leica Microsystems*

Step type: Wash Inc. (min): 0:10 Temperature: Ambient Dispense type: 150 µL

Step Reagent

3. *Bond Wash Solution

Supplier: *Leica Microsystems*

Step type: Wash Inc. (min): 0:10 Temperature: Ambient Dispense type: 150 µL

Step Reagent

4. Cell IDx Block solution or 3% Normal Rabbit Serum

Supplier: *User (Cell IDx provided)*

Step type: Reagent Inc. (min): 20:00 Temperature: Ambient Dispense type: 150 µL

Step Reagent

5. Primary Antibody

Supplier: *User (Cell IDx provided)*



Step type: Reagent Inc. (min): 60:00 Temperature: Ambient Dispense type: 150 μ L

Step Reagent

6. *Bond Wash Solution

Supplier: Leica Microsystems

Step type: Wash Inc. (min): 3:00 Temperature: Ambient Dispense type: 150 μ L

Step Reagent

7. *Bond Wash Solution

Supplier: Leica Microsystems

Step type: Wash Inc. (min): 3:00 Temperature: Ambient Dispense type: 150 μ L

Step Reagent

8. *Bond Wash Solution

Supplier: Leica Microsystems

Step type: Wash Inc. (min): 3:00 Temperature: Ambient Dispense type: 150 μ L

Step Reagent

9. Secondary Antibody

Supplier: User (Cell IDx provided)

Step type: Reagent Inc. (min): 60:00 Temperature: Ambient Dispense type: 150 μ L

Step Reagent

10. *Bond Wash Solution

Supplier: Leica Microsystems

Step type: Wash Inc. (min): 3:00 Temperature: Ambient Dispense type: 150 μ L

Step Reagent

11. *Bond Wash Solution

Supplier: Leica Microsystems

Step type: Wash Inc. (min): 0:00 Temperature: Ambient Dispense type: Open

Step Reagent

12. *Bond Wash Solution

Supplier: Leica Microsystems

Step type: Wash Inc. (min): 3:00 Temperature: Ambient Dispense type: 150 μ L

Step Reagent

13. *Bond Wash Solution

Supplier: Leica Microsystems

Step type: Wash Inc. (min): 0:00 Temperature: Ambient Dispense type: 150 μ L

Step Reagent

14. *Bond Wash Solution

Supplier: Leica Microsystems

Step type: Wash Inc. (min): 0:00 Temperature: Ambient Dispense type: 150 μ L

Step Reagent

15. *Bond Wash Solution

Supplier: Leica Microsystems

Step type: Wash Inc. (min): 0:30 Temperature: Ambient Dispense type: 150 μ L

Step Reagent

16. *Bond Wash Solution

Supplier: Leica Microsystems

Step type: Wash Inc. (min): 0:30 Temperature: Ambient Dispense type: 150 μ L

Step Reagent

17. *Bond Wash Solution

Supplier: Leica Microsystems

Step type: Wash Inc. (min): 0:30 Temperature: Ambient Dispense type: 150 μ L

- 2) Apply 1-3 drops of mounting medium, suggest using Fluoroshield with DAPI (ImmunoBioSciences, Inc, Cat # AR-6501-01) to each slide and then apply coverslip after



incubating 3-5 minutes in the dark at room temperature. **DO NOT USE** Vector VectaShield Mounting Reagent – cat # H-1500

- 3) Allow slides to dry.
- 4) Image slides. Optimal exposure times and gain settings should be determined by the user. For further imaging time considerations, see “Notes” section.

Troubleshooting

| Issue | Possible Cause(s) | Solution |
|--------------------------|----------------------------------|--|
| No antigen signal | Tissue is negative for antigen | Include known positive control tissue in experimental design |
| | Imaging settings are not optimal | Adjust settings using positive control tissue |
| | Antibody did not bind | Always use freshly diluted antibody cocktails |
| High background | Blocking incomplete | Always use freshly prepared blocking buffer and IgG-free BSA |
| | Tissue autofluorescence | Autofluorescence is caused by formaldehyde used for fixation of FFPE tissue and is a common artifact in FFPE based experiments. If autofluorescent background is a significant concern, please contact Cell IDx. |
| Tissue damaged | Antigen retrieval pH < 6.0 | Check pH of antigen retrieval solution |
| | Antigen retrieval time > 30 min | Incubate in antigen retrieval buffer no longer than 30 min total |
| | Tissue poorly affixed to slide | Use positively charged glass slides (e.g. SuperFrost Plus) |
| | Tissue damaged by handling | Gently wash and rinse slides. If using rotator, use low speed |
| | Tissue damaged by handling | Do not allow slides to come in contact with each other Use caution applying coverslip and do not adjust during drying. |

Disclaimer

For Research Use Only. Not for Diagnostic or Therapeutic use.

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