

## Online Data Supplement

### **Specific *In Situ* Immuno-imaging of Pulmonary-Resident Memory Lymphocytes in Human Lungs**

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***T<sub>RM</sub>/B<sub>RM</sub> Immunofluorescence Staining:*** Frozen lung sections were inserted into the Bond-Rx Multiplex IHC Stainer (Leica). Sections were stained according to the following protocol (using 150µL reagent volumes at ambient temperature unless otherwise stated).

1. Universal protein block (30 minutes)
2. Universal peroxidase block
3. Universal peroxidase block (10 minutes)
4. Peroxide block
5. Primary A (CD4 or CD20) (30 minutes)
6. Universal secondary (30 minutes)
7. Tyramide 520 (10 minutes)
8. Heat wash pH9
9. Heat wash pH9
10. Heat wash (85°C, 20 minutes)
11. Universal protein block (30 minutes)
12. Universal peroxidase block

13. Universal peroxidase block (10 minutes)
14. Peroxide block
15. Primary B (CD8 or CD27) (30 minutes)
16. Universal secondary (30 minutes)
17. Tyramide 570 (10 minutes)
18. Heat wash pH9
19. Heat wash pH9
20. Heat wash (85°C, 20 minutes)
21. Universal protein block (30 minutes)
22. Universal peroxidase block
23. Universal peroxidase block (10 minutes)
24. Peroxide block
25. Primary C (CD69) (30 minutes)
26. Universal secondary (30 minutes)
27. Tyramide 620 (10 minutes)
28. Heat wash pH9
29. Heat wash pH9
30. Heat wash (85°C, 20 minutes)
31. For T<sub>RM</sub> panel only - Universal protein block (30 minutes)
32. Universal peroxidase block
33. Universal peroxidase block (10 minutes)
34. Peroxide block
35. Primary D (CD103) (30 minutes)
36. Universal secondary (30 minutes)

37. Tyramide 690 (10 minutes)

38. Heat wash pH9

39. Heat wash pH9

40. Heat wash (85°C, 20 minutes)

41. DAPI counterstain

42. Following wash step, cells mounted using PermaFluor™ (Fisher Scientific)

Slides were scanned using an Axio Scan Z1 (Zeiss, Germany) or imaged using or a Axio Imager Z1 fluorescence microscope (Zeiss).