

May 21, 2025

CD8 Immunohistochemistry Staining

DOI

<https://dx.doi.org/10.17504/protocols.io.kxygxqx54v8j/v1>

Michael Fotheringham¹, Logan L Engle²

¹Queen's University; ²Johns Hopkins University



Michael Fotheringham

Queen's University

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.kxygxqx54v8j/v1>

Protocol Citation: Michael Fotheringham, Logan L Engle 2025. CD8 Immunohistochemistry Staining. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.kxygxqx54v8j/v1>

License: This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working

Created: May 10, 2025



Last Modified: May 21, 2025

Protocol Integer ID: 218008

Keywords: cd8 immunohistochemistry, leica bond rx user manual, leica bond rx, staining slide staining, slide staining, immunohistochemistry

Abstract

Slide staining is performed on the Leica BOND RX. For detailed instructions, refer to the Leica BOND RX user manual. General steps and user-configurable parameters are outlined here for reproducibility.

Materials

- 4- μ m tissue sections
- Leica BOND RX (Leica Biosystems, Buffalo Grove, IL, USA; RRID:SCR_025548)
- Bond Polymer Refine Kit (DS9800, Leica Biosystems; RRID:AB_2891238)
- Anti-CD8 clone 4B11 (CD8-4B11-L-CE, Leica Biosystems; RRID:AB_10555292)
- EcoMount (5082832, Biocare Medical, Walnut Creek, CA, USA)



CD8 Immunohistochemistry Staining

2h 45m

- 1 Obtain 4- μ m sections of each tissue block.
- 2 **Slide staining is performed on the Leica BOND RX (Leica Biosystems, Buffalo Grove, IL, USA; RRID:SCR_025548) using the Bond Polymer Refine Kit (DS9800, Leica Biosystems; RRID:AB_2891238).**

Consult the user manual for your Leica machine for detailed instructions. User-configured parameters are detailed below:
 - 2.1 Select Leica's on-line Bake and Dewax protocol.
 - 2.2 Select high pH EDTA buffer antigen retrieval for 20 minutes at 100°C.
 - 2.3 Select endogenous peroxidase blocking using the peroxidase block included in the Bond Polymer Refine Kit (Leica Biosystems).
 - 2.4 **Select anti-CD8 clone 4B11 (CD8-4B11-L-CE, Leica Biosystems; RRID:AB_10555292) for 15 minutes at a concentration of 19.2 μ g/mL at room temperature.**
 - 2.5 Select counterstain with hematoxylin included in the Bond Polymer Refine Kit (Leica Biosystems).
 - 2.6 Initiate the staining protocol on the Leica BOND RX (Leica Biosystems).
- 3 Remove slides from the Leica Bond RX and dehydrate through graded-alcohol. Coverslip using EcoMount (5082832, Biocare Medical, Walnut Creek, CA, USA).

2h 30m

15m



Protocol references

Taube JM, Akturk G, Angelo M, Engle EL, Gnjjatic S, Greenbaum S, Greenwald NF, Hedvat CV, Hollmann TJ, Juco J, Parra ER, Rebelatto MC, Rimm DL, Rodriguez-Canales J, Schalper KA, Stack EC, Ferreira CS, Korski K, Lako A, Rodig SJ, Schenck E, Steele KE, Surace MJ, Tetzlaff MT, von Loga K, Wistuba II, Bifulco CB; Society for Immunotherapy of Cancer (SITC) Pathology Task Force. The Society for Immunotherapy of Cancer statement on best practices for multiplex immunohistochemistry (IHC) and immunofluorescence (IF) staining and validation. *J Immunother Cancer*. 2020 May;8(1):e000155. doi: 10.1136/jitc-2019-000155. Erratum in: *J Immunother Cancer*. 2020 Jun;8(1):e000155corr1. doi: 10.1136/jitc-2019-000155corr1. PMID: 32414858; PMCID: PMC7239569.

Acknowledgements

Logan L Engle, Michael Fotheringham