

## SOP 13.3 CD3

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 Effective Date: 01-01-2017  
 Last Edited Date: 01-01-2017

### Materials

Name	Source/Vendor	Catalog#	LOT# (optional)
Bond Polymer Refine Detection System	Leica Biosystems	DS9800	
Anti-CD8			

### Buffer and Media

Bond Primary Antibody Diluent, Leica, cat # AR9352

### Protocol

**Antibody clone: CD3 (SP7)**

**Company: Abcam, Cat # ab16669**

Staining for Chromogenic IHC is performed using an automated staining system (BOND RX, Leica Biosystems, Buffalo Grove, IL) following the manufacturer's protocols. All reagents were components of the Bond Polymer Refine detection system (Leica Biosystems)

CD8 immunostaining was performed with a 1:100 dilution using Bond Primary Antibody Diluent (Leica Biosystems).

1. 4- $\mu$ m thick formalin-fixed, paraffin-embedded sections were pre-baked in 60 °C for one hour.
2. Adhesive labels for each protocol were printed and applied to slides.
3. Slides were then loaded onto Bond III with "Bond Universal Covertiles" (Leica Biosystems).
4. Slides were first dewaxed and rehydrated.
5. Heat induced antigen retrieval was performed using ER2 solution (pH8) (Leica Biosystems) for 30 minutes.
6. Primary antibody was incubated for 30 minutes, followed by
  - 10 minutes of post primary blocking reagent,

- 10 minutes of horseradish peroxidase-labeled polymer,
  - 5 minutes of peroxidase block, and
  - 10 minutes of 3,3'-diaminobenzidine developing.
7. Step 7. Slides were counterstained by hematoxylin for 5 minutes.
  8. Step 8. Slides were then taken off from the autostainer, dehydrated and coverslipped.