

# Detection of CD3 in Frozen Mouse Tissue

## Reagent and Antibody Information

[Rapid Fixx](#)

[1X Wash Buffer](#)

[3% Hydrogen Peroxide](#)

[1% BSA Diluent](#)

[Normal Rabbit IgG – Affinity Purified](#)

[DAB Chromogen](#)

[Hematoxylin](#)

Blocking Solution: Rodent Block M (Ready-To-Use)

Biocare Medical

Concord, CA 94520

[www.biocare.net](http://www.biocare.net)

1-800-799-9499

Catalog # RBM961

Primary Antibody: Rabbit Anti-CD3 Polyclonal Antibody

Abcam, Inc

Cambridge, MA 02139

[www.abcam.com](http://www.abcam.com)

1-888-772-2226

Catalog # ab5690

**Lot # GR1047392**

Polymer Reagent: Rabbit-on-Rodent HRP-Polymer Detection

Biocare Medical

Concord, CA 94520

[www.biocare.net](http://www.biocare.net)

1-800-799-9499

Catalog # RMR622

## Staining Procedure

Positive Control Tissue: Spleen and thymus – T-cells

Stain Localization: Cell membrane

1. Cut each frozen section at 5 $\mu$ m and mount on a positively charged slide.  
Immediately fix the section in Rapid Fixx solution for 7 seconds.  
Rinse the slide thoroughly in tap water to remove excess fixative, and then place it in 1X wash buffer.  
Once all the slides have undergone this process, proceed to step 2.
2. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.
3. Quench endogenous peroxidase by placing the slides in 0.3% hydrogen peroxide for 30 minutes.
4. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.
5. Block with the Rodent Block M reagent for 20 minutes at room temperature.  
Lot # \_\_\_\_\_ Exp. Date \_\_\_\_\_

DO NOT RINSE SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.  
ONLY WIPE EXCESS BLOCK.

6. Apply primary antibody at a 1:100 dilution. Incubate for 1 hour at room temperature.  
Lot # \_\_\_\_\_ Exp. Date \_\_\_\_\_

For negative control slides, dilute normal rabbit IgG so that its IgG protein concentration matches that of the primary antibody (if necessary). Then make a 1:100 dilution. If the concentrations can't be matched using this method, the dilution for the negative reagent may need to be adjusted. Apply the negative and incubate for 1 hour at room temperature.

Lot # \_\_\_\_\_ Exp. Date \_\_\_\_\_

7. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.
8. Apply the Rabbit-on-Rodent HRP-Polymer reagent, and incubate for 30 minutes at room temperature.  
Lot # \_\_\_\_\_ Exp. Date \_\_\_\_\_
9. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.
10. Apply the DAB chromogen. Incubate in the dark for 6 minutes at room temperature.  
(Add 1 drop of DAB per ml of substrate)  
Lot # \_\_\_\_\_ Exp. Date \_\_\_\_\_ New Kit: yes / no
11. Rinse the slides in tap water 3 minutes.
12. Counterstain with hematoxylin for 20 seconds.
13. Rinse the slides in tap water until water is clear.
14. Gently agitate slides in 1X wash buffer until the tissues turn blue.

15. Dehydrate through the following solutions:

| <b>Solutions</b> | <b>Repetitions</b> | <b>Time</b> |
|------------------|--------------------|-------------|
| 95% Ethanol      | 1 time             | 3 minutes   |
| 100% Ethanol     | 3 times            | 3 minutes   |
| Xylene           | 2 times            | 5 minutes   |

16. Coverslip

*Updated 01/16/14*