Immunofluorescence Detection of CD3 in Frozen Mouse Tissue

**Reagent and Antibody Information**

1X Wash Buffer  
0.3% Hydrogen Peroxide  
1% BSA Diluent  
**Normal Rabbit IgG – Affinity Purified**  
**ProLong Gold Mounting Media**

Blocking Serum: Normal Goat Serum  
Jackson Immunoresearch Laboratories, Inc.  
West Grove, PA 19390  
www.jacksonimmuno.com  
1-800-367-5296  
Catalog # 005-000-121

**Avidin / Biotin Blocking Kit**  
Vector Laboratories, Inc.  
Burlingame, CA 94010  
www.vectorlabs.com  
1-800-227-6666  
Catalog # SP-2001

**Primary Antibody: Rabbit Anti-CD3 Polyclonal Antibody**  
Abcam, Inc  
Cambridge, MA 02139  
www.abcam.com  
1-888-772-2226  
Catalog # ab5690

**Secondary Antibody: Alexa Fluor® 488 Goat Anti-Mouse IgG (H+L) *Highly Cross-Adsorbed***  
Life Technologies / Invitrogen  
Grand Island, NY 14072  
www.invitrogen.com  
1-888-584-8929  
Catalog # A-11209
**Staining Procedure**

Positive Control Tissue: Spleen – Cytotoxic T-cell lymphocytes  
Stain Localization: Cell membrane and cytoplasmic

1. Cut each frozen section at 6µm and mount on a positively charged slide.  
   Immediately fix the section in Rapid Fix Solution for 7 seconds.  
   Rinse the slide thoroughly in tap water to remove excess fixative and then place 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 10% normal goat serum for 20 minutes at room temperature.  
   Lot #_______________ Date Reconstituted_________________  
   DO NOT RINSE SECTIONS WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.  
   ONLY WIPE EXCESS BLOCK.

6. Apply the 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 it’s 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.  

**From this point on, please perform the stain in the dark.**

8. Apply the Alexa 488 goat anti-mouse secondary antibody at a 1:300 dilution. Incubate for 1 hour at  
   room temperature.  
   Lot #______________ Exp. Date _______________  

9. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.

10. Rinse the slides in tap water.

11. Coverslip with ProLong Gold Mounting Media (with or without DAPI).

12. Store slides at 4°C

*Updated 06/2010*