Detection of CD45R/B220 in Frozen Mouse Tissue

Reagent and Antibody Information

Rapid Fixx
1X Wash Buffer
0.3% Hydrogen Peroxide
1% BSA Diluent
DAB Chromogen
Hematoxylin

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: Rat Anti-Mouse CD45R/B220 Monoclonal Antibody
BD Biosciences
San Jose, CA 95131
1-855-236-2772
www.bdbiosciences.com
Catalog # 550286

Negative Control Serum: Purified Rat IgG2a Isotype Control Serum
BD Biosciences
San Jose, CA 95131
www.bdbiosciences.com
1-855-236-2772
Catalog # 559073

Secondary Antibody: Biotin Polyclonal Goat Anti-Rat Ig (Multiple Adsorbed)
BD Biosciences
San Jose, CA 95131
www.bdbiosciences.com
1-855-236-2772
Catalog # 559286

Label Complex: Peroxidase-Conjugated Streptavidin SS Label
Biogenex Laboratories
San Ramon, CA 94583
www.biogenex.com
1-800-421-4149
Catalog # HK330-9K

Staining Procedure

Positive Control Tissue: Spleen – B-cells
Stain Localization: Membrane and cytoplasmic

1. Cut each frozen section at 6µm 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 5% normal goat serum for 20 minutes at room temperature.
   Lot #_____ Date Reconstituted_____

DO NOT RINSE SLIDES. CONTINUE TO AVIDIN-BIOTIN BLOCK.

6. Avidin / Biotin Blocking Kit
   Lot #_____ Exp. Date_____New Kit: yes / no
   Apply avidin block for 15 minutes at room temperature.
   Quick rinse in 1X wash buffer.
   Apply biotin block for 15 minutes at room temperature.

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

7. Apply primary antibody at a 1:1000 dilution. Incubate for 1 hour at room temperature.
   Lot #_____ Exp. Date_____

   For negative control slides, dilute rat IgG2a control serum so that it's IgG2a protein concentration matches that of the primary antibody (if necessary). Then make a 1:1000 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 _____

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

9. Apply the goat anti-rat Ig secondary antibody at a 1:200 dilution. Incubate for 30 minutes at room

temperature.
Lot #_____ Exp. Date_____

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

11. Apply the Streptavidin SS Label. Incubate for 30 minutes at room temperature.
    Lot #_____ Exp. Date _____

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

13. 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

14. Rinse the slides in tap water 3 minutes.

15. Counterstain with hematoxylin for 20 seconds.

16. Rinse the slides in tap water until water is clear.

17. Gently agitate slides in 1X wash buffer until the tissue turns blue.

18. Dehydrate through the following solutions:

Solutions	Repetitions	Time
95% Ethanol	1 time	3 minutes
100% Ethanol	3 times	3 minutes
Xylene	2 times	5 minutes

19. Coverslip

Updated 11/08/10