

Detection of CD21/CD35 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 Rabbit Serum

Jackson ImmunoResearch Laboratories, Inc.

West Grove, PA 19390

www.jacksonimmuno.com

1-800-367-5296

Catalog # 011-000-001

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 CD21/CD35 Monoclonal Antibody

BD Biosciences

San Jose, CA 95131

1-855-236-2772

www.bdbiosciences.com

Catalog # 553817

Lot # 3011764

Negative Control Serum: Purified Rat IgG2b Isotype Control Serum

BD Biosciences

San Jose, CA 95131

www.bdbiosciences.com

1-855-236-2772

Catalog # 559478

Secondary Antibody: Biotinylated Rabbit Anti-Rat IgG (H+L)

Vector Laboratories, Inc.

Burlingame, CA 94010

www.vectorlabs.com

1-800-227-6666

Catalog # BA-4001

Label Complex: R.T.U. Vectastain Elite ABC Reagent

Vector Laboratories, Inc.

Burlingame, CA 94010

www.vectorlabs.com

1-800-227-6666

Catalog # PK-7100

Staining Procedure

Positive Control Tissue: Spleen – peripheral B-lymphocytes

Stain Localization: Membrane

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 rabbit 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 1:50 dilution. Incubate for 1 hour at room temperature.
Lot # _____ Exp. Date _____

For negative control slides, dilute rat IgG2b control serum so that it's IgG2b protein concentration matches that of the primary antibody (if necessary). Then make a 1:50 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 rabbit anti-rat secondary antibody at a 1:500 dilution. Incubate for 30 minutes at room temperature.

Lot # _____ Date Reconstituted _____

10. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.
11. Apply the Vectastain R.T.U. Elite Label and incubate for 30 minutes at room temperature.
Exp. Date _____ New Kit: yes / no
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 tissues turn 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 01/16/14