Detection of CD19 in Frozen Mouse Tissue

Reagents:
1X Automation Buffer
.3% Hydrogen Peroxide
Antibody Diluent
DAB Chromagen
Hematoxylin
Rapid Fixx

Antibody Information

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 CD19
Serotec, Inc.
Raleigh, NC 27604
1-800-265-7376
www.serotec-inc.com
Catalog# MCA1439GA

Negative control: Purified Rat IgG 2a
BD Pharmingen
Distributed by Transduction Labs
Lexington, KY 40511
www.bdpharma.com
1-800-227-4063
Catalog# 559073

Secondary Antibody: Biotin Polyclonal Goat anti-rat Ig (multiple adsorbed)
BD Pharmingen
Distributed by Transduction Labs
Lexington, KY 40511
www.bdpharma.com
1-800-227-4063
Catalog #5590286

Label antibody: StriAviGen Super Sensitive Predilute Label Antibody
Biogenex Laboratories
San Ramon, CA 94583
www.biogenex.com
1-800-421-4149
Catalog #HK330-5K

Staining Procedure

-Positive Control Tissue: Frozen spleen
-Stain localization: cytoplasm – cell membrane

For Frozen Tissue Sections
Six micron sections are cut and immediately fixed in Rapid Fix (Shandon-Lipshaw) for 7 seconds. Place section in 1X AB until all sections are cut. After the last section is cut, rinse slides in 1X AB for 5 minutes. Repeat buffer rinse.

1. Quench endogenous peroxidase by placing slides in 0.3% hydrogen peroxide for 30 minutes.

2. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

3 Apply 5% Normal Goat Serum and incubate for 20 minutes at room temperature.
Lot#_____ Reconstituted_____

Wipe excess reagent from around tissue section. DO NOT RINSE SECTIONS WITH BUFFER.

4. Apply Avidin/Biotin block
Lot#_____ Exp Date_____ New Kit: yes / no
Apply avidin block - 15 min at RT.
Quick rinse in 1X AB.
Apply biotin block - 15 min at RT.
Wipe excess block.

DO NOT RINSE SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.

5. Apply primary antibody CD19 at a 1:500 dilution and incubate for one hour at room temperature.
Lot#_____ Exp Date_____

For the negative control slides, normalize the concentration of purified Rat IgG-2a negative control serum with the protein concentration of the CD19 antibody. Apply to slides at a 1:500 dilution and incubate for one hour.
Lot#_____ Exp Date_____

6. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

7. Apply secondary antibody (Goat anti-rat IgG) at a 1:200 dilution and incubate for 30 minutes at room temperature.
Lot#_____ Exp Date_____

8. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

9. Apply Label antibody (StriAviGen Super Sensitive Predilute) and incubate for 30 minutes at room temperature.
Lot #_____ Exp. Date _____

10. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

11. Apply liquid Dako DAB Chromagen for 6 minutes in the dark.
(Add 1 drop of DAB per ml of substrate)
Lot#_____ Exp. Date_____ New Kit: yes / no

12. Rinse in tap water 3 minutes.

13 Counterstain with Modified Harris Hematoxylin for 30 seconds.

14 Rinse in tap water until water is clear.

15 Place slides in 1X Automation buffer for one minute with gentle agitation to blue slides.

16 Dehydrate through the following solutions.

95% alcohol	1 times	3 mins
100% alcohol	3 times	3 mins
Xylene	2 times	5 mins

17. Coverslip
updated 05/13/05