Detection of Pax-5 in Frozen Mouse Tissue

Reagent and Antibody Information

Rapid Fixx
1X Wash Buffer
0.3% Hydrogen Peroxide
1% BSA Diluent
1X Citrate Buffer
Normal Goat IgG – Affinity Purified
DAB Chromogen
Hematoxylin

Blocking Serum: Normal Horse Serum
Jackson Immunoresearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296
Catalog # 008-000-001

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

Primary Antibody: Goat Polyclonal Pax-5 Antibody (C-20)
Santa Cruz Biotechnology
Santa Cruz, CA 95060
www.scbt.com
1-800-457-3801
Catalog # sc-1974

Secondary Antibody: Biotinylated Horse Anti-Goat IgG (H+L)
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog # BA-9500

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: Nuclear

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 slides in 0.3% hydrogen peroxide for 30 minutes.

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

4. Heat-Induced Epitope Retrieval Using The Decloaker
   Add 500 ml of distilled water to the pan inside the decloaker.
   Place a full rack of slides into a Tissue Tek® container with 200 ml of 1X citrate buffer
   (Insert blank slides into any empty slots in the rack to ensure even heating of slides)
   Place the container stably inside the pan and decloak for 5 minutes. *Maximum Pressure* _____
   Depressurize for 10 minutes.
   Remove pan top and cool for 10 minutes. *Temperature Before Cooling Slides*_____
   Rinse the slides in 2 changes of distilled water for 3 minutes each time.

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

6. Block with 10% normal horse serum for 20 minutes at room temperature.
   Lot #_____ Date Reconstituted_____

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

7. 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 SECTIONS WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.
   ONLY WIPE EXCESS BLOCK.

8. Apply primary antibody at a 1:250 dilution. Incubate for 1 hour at room temperature.
   Lot #_____ Exp. Date_____

   For negative control slides, dilute normal goat IgG so that it's IgG protein concentration matches that of the primary antibody (if necessary). Then make a 1:250 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 _____

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

10. Apply the horse anti-goat secondary antibody at a 1:1000 dilution. Incubate for 30 minutes at room temperature.
    Lot #_____ Date Reconstituted_____

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

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

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

14. 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

15. Rinse the slides in tap water 3 minutes.

16. Counterstain with hematoxylin for 20 seconds.

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

18. Gently agitate slides in 1X wash buffer until the tissues turn blue.

19. 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

20. Coverslip

Updated 11/06/09

This protocol has been made available for use by others. We cannot guarantee optimal results, so staining conditions must be determined by the end user.
Protocol modifications may be required due to lot number changes and/or reagent substitutions.