Detection of S100 in Formalin-Fixed, Paraffin-Embedded in Mouse Tissue

Reagents:

1X Automation Buffer
3% Hydrogen Peroxide
Antibody Diluent
Citrate Buffer
DAB Chromagen
Hematoxylin

Antibody Information

Blocking Serum: Normal Goat Serum
Jackson Immunoresearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296
Catalog #005-000-001

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

Primary antibody: Rabbit anti-S100
Neomarkers
Fremont, CA 94539
www.labvision.com
1-800-828-1628
Catalog# RB-044-A1

Negative Control: Normal Rabbit Serum
Jackson Immunoresearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296
Catalog #011-000-101

Secondary antibody: Biotinylated Goat anti-rabbit IgG
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog #BA-1000

Label antibody: Vector Standard Elite Kit
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog #PK-6100

Staining Procedure

-Positive Control Tissue: mouse brain, Schwannoma
-Stain Localization: Cytoplasmic

Deparaffinize and hydrate slides through the following solutions.

Xylene	2 times	5 minutes
100% EtOH	2 times	3 minutes
95% EtOH	2 times	3 minutes
1X Automation Buffer	2 times	5 minutes

1. Quench endogenous peroxidase by placing slides in 3% hydrogen peroxide for 15 minutes.

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

3. Block in 5% Normal Goat Serum for 20 minutes.
Lot#_____ Reconstitution Date_____

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 reagent from around tissue section.

DO NOT RINSE SECTIONS WITH BUFFER.

7. Apply primary antibody (Rabbit anti-S100) at 1:300 dilution and incubate for 30 min.
Lot#_____ Exp Date_____

For the negative control slides, normalize the protein concentration of the normal rabbit serum to the protein concentration of the primary antibody (S100). Use this to make the 1:150 dilution and incubate for 30 min.
Lot #_____ Reconstituted Date_____

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

9. Apply secondary antibody (Goat anti-rabbit) at a 1:500 dilution and incubate for 30 minutes.
Lot#_____ Reconstituted Date_____

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

11. Apply Label antibody from Vector Standard Elite Kit and incubate for 30 minutes.
(Prepare at least 30 mins prior to use)
Exp. Date_____ New Kit: yes / no

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

13. 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

14. Rinse in tap water 3 minutes.

15. Counterstain with Modified Harris Hematoxylin for 30 seconds.

16. Rinse in tap water until water is clear.

17. Place slides in 1X Automation Buffer for 1 minute with gentle agitation to blue slides.

18. Dehydrate through the following solutions.

95% Ethanol	1 change	3 minutes
100% EtOH	3 changes	3 minutes
Xylene	2 changes	5 minutes

19. Coverslip

updated 09/29/04