

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

Reagents:

[1X Automation Buffer](#)
[3% Hydrogen Peroxide](#)
[Antibody Diluent](#)
[0.05% Pronase](#)
[DAB Chromagen](#)
[Hematoxylin](#)

Antibody Information:

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

Dako Protein Block Serum-Free--Ready to use
Dako Corporation
Carpinteria CA
www.dakousa.com
1-800-235-5763
Catalog # X0909

Primary antibody: Cow Glial Fibrillary Acidic Protein (rabbit anti-GFAP)
Dako Corporation
Carpinteria, CA
www.dakousa.com
1-800-235-5763
Catalog #Z0334

Negative control serum: Normal Rabbit Serum
Jackson Immunoresearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296
Catalog #011-000-001

Kit: Dako LSAB+ System HRP

Dako Corporation

Carpinteria CA

www.dakousa.com

1-800-235-5763

Cat # K06901

Pronase Solution

According to manufacturer's instructions

Dako Corporation

Carpinteria CA 93013

www.dakousa.com

1-800-235-5763

Catalog #S2013

Staining Procedure

-Positive Control Tissue: Mouse Brain (glial cells)

-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. Perform Heat Induced Epitope Retrieval using 0.05% Pronase
Lot # _____
(Dilute 100ul stock in 1.9ml 1X Automation Buffer)
Incubate slides in 0.05% Pronase for 2 minutes in oven at 37 degrees.
Rinse slides in distilled water for 1 minute to stop digestion.
4. Rinse slides in 2 changes of 1X automation buffer for 5 minutes

5. Block in Dako Protein Block Serum-Free for 10 minutes at room temperature.

Lot# _____ Exp. Date _____

DO NOT RINSE CONTINUE TO A/B BLOCK

6. 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.

DO NOT RINSE SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.

7. Apply primary antibody (GFAP) at a 1:1500 dilution and incubate for 30 minutes at room temperature.

Lot# _____ Exp. Date _____

For negative control slides, normalize the protein concentration of normal rabbit serum to the protein concentration of the primary antibody (GFAP) and use this to make the 1:1500 dilution. Apply normal rabbit serum to the slides and incubate for 30 min at room temperature.

Lot# _____ Reconstituted Date _____

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

LSAB+ Kit Lot# _____ Exp. Date _____

9. Apply link – secondary (yellow bottle) and incubate for 15 minutes at room temperature.

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

11. Apply label antibody (red bottle) incubate for 15 minutes at room temperature.

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 11/22/05