

# Detection of FAS-L in Formalin-Fixed, Paraffin-Embedded Mouse Tissue

## Reagents:

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

## Antibody Information:

Kit: Rabbit Elite kit

Vector Laboratories, Inc.  
Burlingame, CA 94010  
[www.vectorlabs.com](http://www.vectorlabs.com)

1-800-227-6666

Catalog: PK-6101

\*The Vector Rabbit Elite Kit contains solutions needed to make the block, secondary and label antibodies.

Avidin Biotin Blocking Kit

Vector Laboratories, Inc.  
Burlingame, CA 94010  
[www.vectorlabs.com](http://www.vectorlabs.com)

1-800-227-6666

Catalog #SP-2001

Primary antibody: Rabbit Anti-FAS-L

Santa Cruz Biotechnology, Inc.  
Santa Cruz, CA 95060  
[www.scbt.com](http://www.scbt.com)

1-800-457-3801

Catalog #sc-834

Negative control serum: Normal Rabbit Serum

Jackson ImmunoResearch Laboratories, Inc.  
West Grove, PA 19390  
[www.jacksonimmuno.com](http://www.jacksonimmuno.com)

1-800-367-5296

Catalog #011-000-001

## Staining Procedure

-Positive Control Tissue: normal mouse brain

-Stain Localization: Cytoplasmic (axons, glial cells, surrounding blood vessels)

Comment: Our protocol was developed from a study of Fas-L staining in the normal rat and human brain. (Bechman et al., GLIA 27:62-74, 1999)

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 one change of 1X Automation Buffer for 5 minutes at room temperature.

3. Perform Heat Induced Epitope Retrieval using a microwave oven  
Unmasking Techniques

Place a full rack of slides in a container containing 200mls 1X citrate buffer.

Microwave for 5 minutes at level 5.

Cool for 1 minute

Microwave again for 5 minutes at level 5. Temp. \_\_\_\_\_

Remove the slides from the microwave oven and allow to cool 20 minutes at room temperature.

Rinse slides in distilled water for 2 times for 2 mins each.

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

5. Apply blocking solution and incubate for 20 minutes at room temperature.

Exp. Date \_\_\_\_\_ New Kit: yes / no

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

Wipe excess block

DO NOT RINSE SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.

7. Apply primary antibody (FAS-L) at a 1:200 dilution and incubate overnight at 4 degrees Celsius.

Lot# \_\_\_\_\_ Exp Date \_\_\_\_\_

For negative control slides, normalize the protein concentration of the normal rabbit serum to the protein concentration of the primary antibody (FasL) and use this to make the 1:200 dilution and incubate overnight at 4 degrees Celsius.

Lot# \_\_\_\_\_ Reconstituted Date \_\_\_\_\_

\*\*\*\*\*Next Day\*\*\*\*\*

Remove slides from refrigerator and allow them to come to room temperature for 30min.

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

9. Apply secondary antibody and incubate for 30 minutes at room temperature.

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

11. Apply Label antibody and incubate for 30 minutes at room temperature.  
(Prepare at least 30 mins prior to use)

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 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 10/12/05