

Detection of Mouse IgG in Formalin Fixed Paraffin Embedded Mouse Tissue

Reagents:

[1X Automation Buffer](#)

[3% Hydrogen Peroxide](#)

[Antibody Diluent](#)

[DAB Chromagen](#)

Hematoxylin

Antibody Information:

Normal Donkey Serum

Jackson ImmunoResearch Laboratories, Inc.

West Grove, PA 19390

www.jacksonimmuno.com

1-800-367-5296

Catalog #017-000-001

Avidin Biotin Blocking Kit

Vector Laboratories, Inc.

Burlingame, CA 94010

www.vectorlabs.com

1-800-227-6666

Catalog #SP-2001

Primary antibody: Goat anti-mouse IgG

Cedarlane Laboratories

Ontario, Canada LOP1EO

1-800-268-5058

www.Cedarlanelabs.com

Catalog #CLCC30200

Concentration: 0.8 mg/ml

Negative control serum: Normal Goat Serum

Jackson ImmunoResearch Laboratories, Inc.

West Grove, PA 19390

www.jacksonimmuno.com

1-800-367-5296

Catalog #005-000-121

Secondary antibody: Donkey anti-goat

Jackson ImmunoResearch Laboratories, Inc.

West Grove, PA 19390

www.jacksonimmuno.com

1-800-367-5296

Catalog #705-065-147

Label antibody: Vector EliteVectastain® ABC
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog #PK-6100

Staining Procedure

- Positive Control Tissue: TCDD treated mouse tissue
- Stain Localization: Membrane

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 Donkey Serum for 20 minutes.
Lot# _____ Reconstituted Date _____
4. Apply Avidin/Biotin block
Lot# _____ Exp date _____ New kit yes / no
Apply avidin block - 15 min @ RT.
Quick rinse in 1X Automation Buffer. (Do not leave slides sitting in buffer)
Apply biotin block - 15 min @ RT.
No wash, wipe excess block and apply primary antibody
DO NOT RINSE SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.
5. Apply primary antibody Goat anti-mouse IgG at 1:250 dilution and incubate one hour.
Lot# _____ Exp Date _____

1:250

For negative control slides, normalize the protein concentration of the normal goat serum to the protein concentration of the primary antibody (goat anti-mouse IgG) and use this to make the dilutions and incubate for one hour.

Lot# _____ Reconstituted Date _____

1:250

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

7. Apply secondary antibody (Donkey anti-goat) at a 1:500 and incubate for 30 minutes.

Lot# _____ Reconstituted Date _____

1:500

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

9. Apply Label antibody from Vector Elite Kit and incubate for 30 minutes. (Prepare 30 minutes prior to use)

Kit Lot# _____ Exp. Date _____ New kit yes / no

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 20 seconds.

14. Rinse in tap water until clear.

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

16. Dehydrate through the following solutions.

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

17. Coverslip

updated 8/8/2003
