

Detection of Thyroglobulin in Formalin-Fixed, Paraffin-Embedded in Rat Tissue

Reagents:

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

Antibody Information

Kit: Vector Mouse Elite Kit

Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com

1-800-227-6666

Catalog: PK6102

*This kit includes reagents needed to make blocking reagent, secondary and label antibodies.

Avidin Biotin Blocking Kit

Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com

1-800-227-6666

Catalog #SP-2001

Primary antibody: Mouse anti-thyroglobulin

Neomarkers

Fremont, CA

www.neomarkers.com

Catalog# MS-1380-P

Negative Control: Normal Mouse Serum

Jackson ImmunoResearch Laboratories, Inc.
West Grove, PA 19390

www.jacksonimmuno.com

1-800-367-5296

Catalog #015-000-001

Staining Procedure

-Positive Control Tissue: rat GI (colon)

-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. Apply blocking solution from the Vector Mouse Elite kit and incubate for 20 minutes.
Exp. Date _____ New Kit: yes / no

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.

5. Apply primary antibody (thyroglobulin) at 1:100 dilution and incubate for 30 min.

Lot# _____ Exp _____

For the negative control slides, normalize the protein concentration of the normal mouse serum to the protein concentration of the primary antibody (thyroglobulin). Use this to make the 1:100 dilution and incubate for 30 min.

Lot # _____ Reconstituted Date _____

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

7. Apply secondary antibody from the Mouse Elite Kit and incubate for 30 minutes.

8. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.
9. Apply Label antibody from Vector Mouse Elite Kit and incubate for 30 minutes.
(Prepare at least 30 mins prior to use)
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 30 seconds.
14. Rinse in tap water until water is 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 03/17/04