

Detection of Estrogen Receptor Alpha in Formalin Fixed, Paraffin-Embedded 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 #PK-6102

*This kit contains all reagents necessary to make blocking, 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-ER (ER1D5)

Immunotech, Inc.

1-800-458-5060

Catalog #1545

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 uterus, vagina or oviduct

-Stain localization: Nuclear

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. Unmasking Techniques using the decloaker.

Add 500 ml D/W to the pan of the decloaker.

Place full rack of slides in 200 ml of 1X citrate buffer and place in the decloaker.

Decloak for 5 minutes. Pressure_____

Depressurize for 10 minutes.

Remove pan top and cool for 10 min.Temp_____

Rinse in D/W, 2x for 3 min each

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

5. Apply blocking solution from Vector Kit for 20 minutes at room temperature.

Exp. Date_____ New Kit:: yes / no

DO NOT RINSE SECTIONS WITH BUFFER.

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 (mouse anti-ER) at 1:300 dilution and incubate for one hour at room temperature.

Lot# _____ Exp Date _____

For negative control slides, normalize the protein concentration of the Normal Mouse Serum to the protein concentration of the primary antibody (Estrogen Receptor) and use this to make the 1:300 dilution. Apply to slides and incubate for one hour at room temperature.

Lot # _____ Reconstituted Date _____

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

9. Apply the secondary antibody and incubate for 30 minutes.

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

11. Apply the label antibody and incubate for 30 minutes.
(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 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
update 09/24/04