Detection of Estrogen Receptor Alpha in Formalin-Fixed, Paraffin Embedded Mouse Tissue

Reagents:

1X Automation Buffer
3% Hydrogen Peroxide
Antibody Diluent
Citrate Buffer
DAB Chromagen
Hematoxylin

Antibody Information

Kit: Vector M.O.M.™ Kit
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog: PK2200
*The Vector M.O.M.™ kit includes reagents needed to make blocking reagent, working diluent, 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: Uterus, vagina, 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. Incubate sections for 1 HOUR in MOM specific IgG blocking reagent
(Made via 2.5 mls diluent plus 2 drops of Mouse IgG blocking reagent)
Lot #_____ Exp. Date_____ New Kit: yes / no

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 reagent from around tissue section. DO NOT RINSE SECTIONS WITH BUFFER.

7. Make primary antibody dilution in Vector MOM diluent. (600ul of protein stock in 7.5 mls PBS)

8. Apply primary antibody (Mouse anti-estrogen receptor) at 1:25 dilution and incubate for one hour.
Lot#_____ Exp_____

For the negative control slides, normalize the protein concentration of the normal mouse serum to the protein concentration of the primary antibody (Estrogen Receptor). Use this to make the 1:25 dilution and incubate for one hour.
Lot #_____ Reconstituted Date_____

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

10. Apply M.O.M. secondary (biotinylated anti-mouse IgG) and incubate for 10 minutes (Made via 10ul of antibody in 2.5mls of Vector MOM diluent).
Lot#_____ Exp Date_____

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

12. Apply Vectastain ABC Elite label for 5 minutes. (Prepare 30 minutes before use)
(Made via 2 drops of Reagent A plus 2 drops of Reagent B in 2.5 mls BSA diluent)
Exp Date_____ New Kit: yes / no

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

14. 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

15. Rinse in tap water 3 minutes.

16. Counterstain with Modified Harris Hematoxylin for 30 seconds.

17. Rinse in tap water until water is clear.

18. Place slides in 1X Automation Buffer for 1 minute with gentle agitation to blue slides.

19. Dehydrate through the following solutions.

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

20. Coverslip

updated 2/4/04