Detection of p63 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

Note: The Vector M.O.M. Kit contains solutions needed to make the block, secondary, and label reagents.

Avidin Biotin Blocking Kit
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog # SP-2001

Primary antibody: Mouse Monoclonal Antibody to p63
Neomarkers / Lab Vision
Fremont, CA
www.neomarkers.com
1-800-828-1628
Catalog # MS-1081-P1

Negative control serum: 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: Papilloma skin
Stain Localization: Nuclear

Deparaffinize and hydrate slides through the following solutions.

Xylene	2 times	5 minutes
100% Ethanol	2 times	3 minutes
95% Ethanol	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 technique using the decloaker.
   Add 500 ml distilled water 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 minutes. Temperature before cooling_____
   Rinse in distilled water twice for 3 minutes each.

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

5. Block with the M.O.M. IgG blocking reagent for one hour at room temperature. Make by adding 2 drops of Mouse IgG blocking reagent to 2.5ml of 1X PBS.
   Kit Lot#_____ Exp Date_____New Kit: yes / no

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

6. Apply the Avidin Biotin Blocking Kit
   Lot#_____ Exp Date_____New Kit: yes / no
   Apply avidin block - 15 minutes at room temperature.
   Quick rinse in 1X Automation Buffer.
   Apply biotin block - 15 minutes at room temperature.
   No wash, wipe excess block and apply primary antibody

   DO NOT RINSE SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.

   Prepare Vector M.O.M .diluent: 600ul of protein concentrate stock in 7.5ml of 1X

PBS. Make primary, secondary, and label antibodies in Vector M.O.M. diluent.

7. Apply primary antibody (p63) at a 1:300 dilution and incubate for 30 minutes at room temperature
   Lot#_____ Exp Date _____

   For negative control slides, normalize the protein concentration of the normal mouse serum to match the protein concentration of the primary antibody (p63) and use this to make a 1:300 dilution. Apply to slides and incubate for 30 minutes at room temperature.
   Lot#_____ Reconstituted Date _____

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

9. Apply M.O.M. biotinylated anti-mouse IgG secondary and incubate for 10 minutes at room temperature. Make by adding 10ul of antibody to 2.5ml of M.O.M. diluent.

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

11. Apply Vector Elite Label for 5 minutes. Prepare at least 30 minutes before use by adding 2 drops of Reagent A and 2 drops of Reagent B to 2.5ml of M.O.M. diluent

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 20 seconds.

16. Rinse in tap water until water is clear.

17. Gently agitate slides in 1X Automation buffer until they turn blue.

18. Dehydrate through the following solutions.

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

19. Coverslip

Updated 12/19/06