Identification of Keratin 15 in Formalin-Fixed, Paraffin Embedded Rat Tissue

Reagents:

1X Automation Buffer

3% Hydrogen Peroxide

Antibody Diluent

DAB Chromagen

Hematoxylin

Antibody Information:

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

Primary antibody: Keratin 15 (Clone LHK15)
Neomarkers/Labvision
Fremont, CA
Catalog #MS-1068-P
www.neomarkers.com
Concentration: 200 ug/ml

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

Dako Protein Block Serum-Free
Dako Corporation
Carpinteria CA 93013
www.dakousa.com
Code no. X0909

Kit: Dako LSAB+ System HRP
DakoCorporation
Carpinteria CA 93013
www.dakousa.com
Code No. K06901

Staining Procedure

-Positive Control Tissue: Rat GI tract (squamous epithelium)
-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. 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.

5. Incubate sections for 10 minutes in DAKO Protein Block Serum-Free at RT.
Lot#_____Exp Date_____

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 (Keratin 15) a 1:15 dilution and incubate for 30 minutes.
Lot#_____Exp Date_____

For negative control slides, normalize the protein concentration of normal mouse serum to the protein concentration of the primary antibody (Keratin 15) and use this to make the 1:15 dilution.
Lot#_____ Reconstituted Date_____

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

9. Apply Link antibody (Yellow bottle) from LSAB+ kit for 15 minutes.
Kit Lot#_____ Exp Date_____

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

11. Apply label antibody (Red bottle) from LSAB+ kit for 15 minutes

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 45 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.

updated 01/10/05