Detection of Cytokeratin 8 in Formalin-Fixed, Paraffin-Embedded Rat Tissue

Reagent and Antibody Information

1X Wash Buffer
3% Hydrogen Peroxide
1% BSA Diluent
Trypsin
DAB Chromagen
Hematoxylin

Staining Kit: Vectastain Elite ABC Kit (Mouse IgG)
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog # PK-6102

Note: This kit contains all reagents necessary to make the blocking solution, secondary antibody and label complex.

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 Cytokeratin 8
Abcam Inc
Cambridge, MA 02139
www.abcam.com
1-888-772-2226
Catalog # ab9287-100

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: Normal skin
Stain Localization: Cytoplasmic

1. 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 Wash Buffer	2 times	5 minutes

2. Quench endogenous peroxidase by placing the slides in 3% hydrogen peroxide for 15 minutes.

3. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each.

4. Proteolytic-Induced Epitope Retrieval Using Trypsin
   Incubate the slides in a 0.01% trypsin solution in a water bath at 37°C for 20 minutes.
   (DO NOT add the trypsin to the 0.05M Tris-HCl • $CaCl_2$ solution until 5 minutes prior to incubation.
   Trypsin looses 75% of its reactivity within 30 minutes at 37°C.)
   Rinse the slides in distilled water for 1 minute to stop the enzymatic digestion.

5. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each time.

   Vectastain Mouse Elite Staining Kit
   Exp Date_____ New Kit: yes / no

6. Apply the blocking solution from the Mouse Elite Kit and incubate for 20 minutes at room temperature.

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

7. Avidin / Biotin Blocking Kit
   Lot #_____ Exp. Date_____New Kit: yes / no
   Apply avidin block - 15 minutes at room temperature.
   Quick rinse in 1X Wash Buffer.
   Apply biotin block - 15 minutes at room temperature.

   DO NOT RINSE SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.
   ONLY WIPE EXCESS BUFFER.

8. Apply primary antibody at a 1:5000 dilution and incubate for 1 hour at room temperature.
   Lot #_____ Exp Date _____

   For negative control slides, dilute the protein concentration of the normal mouse serum to match that of the primary antibody. Make a 1:5000 dilution from this normalized serum and apply to the slides. Incubate for 1 hour at room temperature.

Lot #_____ Date Reconstituted_____

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

10. Apply the secondary antibody from Mouse Elite Kit and incubate for 30 minutes at room temperature.

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

12. Apply the label complex from the Mouse Elite Kit and incubate for 30 minutes at room temperature.

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

14. Apply the DAB chromagen and incubate in the dark for 6 minutes at room temperature.
(Add 1 drop of DAB per ml of substrate)
Lot #_____ Exp Date_____ New Kit: yes / no

15. Rinse the slides in tap water 3 minutes.

16. Counterstain with Harris Hematoxylin for 30 seconds.

17. Rinse the slides in tap water until water is clear.

18. Gently agitate slides in 1X Wash Buffer until they turn blue.

19. Dehydrate through the following solutions:

95% Ethanol	1 time	3 minutes
100% Ethanol	3 times	3 minutes
Xylene	2 times	5 minutes

20. Coverslip

Updated 03/16/07