Detection of Cytokeratin 15 in Formalin-Fixed, Paraffin-Embedded Mouse Tissue

Reagent and Antibody Information

1X Wash Buffer
3% Hydrogen Peroxide
1% BSA Diluent
1X Citrate Buffer
Normal Rabbit IgG – Affinity Purified
DAB Chromogen
Hematoxylin

Staining Kit: Vectastain Elite ABC Kit (Rabbit IgG)
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog # PK-6101

Note: This kit contains all reagents necessary to make the blocking reagent, 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: Rabbit Anti-Human KRT15 Polyclonal Antibody
Proteintech Group, Inc
Chicago, IL 60612
www.ptglab.com
1-888-478-4522
Catalog # 10137-1-AP

Staining Procedure

Positive Control Tissue: Skin – epidermis, sweat glands, and epithelial cells of hair follicles
Stain Localization: Cytoplasmic

1. Deparaffinize and hydrate slides through the following solutions:

Solution	Repetitions	Time
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. Heat-Induced Epitope Retrieval Using The Microwave
   Place a full rack of slides into a Tissue Tek® container with 200 ml of 1X citrate buffer
   (Insert blank slides into any empty slots in the rack to ensure even heating of slides)
   Microwave for 5 minutes at power level 5.
   Cool for 1 minute. (Add more citrate buffer, if necessary.)
   Microwave again for 5 minutes at power level 5. *Temperature Before Cooling Slides*_____
   Cool 20 minutes at room temperature.
   Rinse the slides in 2 changes of distilled water for 3 minutes each time.

5. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each time.

   Vectastain Rabbit Elite Staining Kit
   Exp. Date_____ New Kit: yes / no

6. Apply the block from the Rabbit Elite Kit. 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 for 15 minutes at room temperature.
   Quick rinse in 1X wash buffer.
   Apply biotin block for 15 minutes at room temperature.

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

8. Apply primary antibody at a 1:800 dilution. Incubate for 1 hour at room temperature.
   Lot #_____ Exp. Date _____

   For negative control slides, dilute normal rabbit IgG so that it's IgG protein concentration matches that

of the primary antibody (if necessary). Then make a 1:800 dilution. If the concentrations can't be matched using this method, the dilution for the negative reagent may need to be adjusted. Apply the negative and incubate for 1 hour at room temperature.
Lot #_____ Exp. Date _____

9. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.

10. Apply the secondary antibody from Rabbit Elite Kit. 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 Rabbit Elite Kit. Incubate for 30 minutes at room temperature. (Prepare at least 30 minutes prior to use.)

13. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.

14. Apply the DAB chromogen. 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 hematoxylin for 20 seconds.

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

18. Gently agitate slides in 1X wash buffer until the tissues turn blue.

19. Dehydrate through the following solutions:

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

20. Coverslip

Updated 08/23/07

This protocol has been made available for use by others. We cannot guarantee optimal results, so staining conditions must be determined by the end user.
Protocol modifications may be required due to lot number changes and/or reagent substitutions.