Detection of Vimentin in Formalin-Fixed, Paraffin-Embedded Rat Tissue

Reagent and Antibody Information

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

Blocking Serum: Normal Horse Serum
Jackson Immunoresearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296
Catalog # 008-000-001

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

Primary Antibody: Goat Anti-Vimentin (Whole Antiserum) Antibody
Sigma-Aldrich
St. Louis, MO
www.sigmaaldrich.com
1-800-325-3010
Catalog # V4630

Negative Control Serum: Normal Goat Serum
Jackson Immunoresearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296
Catalog # 005-000-121

Secondary Antibody: Biotinylated Horse Anti-Goat IgG (H+L)
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog # BA-9500

Label Complex: Vectastain Elite ABC Kit (Standard)
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog # PK-6100

Staining Procedure

Positive Control Tissue: Kidney (glomeruli and endothelial cells in the medulla)
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. Proteolytic-Induced Epitope Retrieval Using Trypsin
   Incubate the slides in a 0.01% trypsin solution in a water bath at 37°C for 30 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.

6. Block with 5% Normal Horse Serum for 20 minutes at room temperature.
   Lot #_____ Date Reconstituted_____

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

8. Apply primary antibody at a 1:1000 dilution, and incubate for 1 hour at room temperature.

Lot #_____ Date Aliquoted_____
For negative control slides, dilute the protein concentration of the normal goat serum to match that of the primary antibody. Make a 1:1000 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 horse anti-goat secondary antibody at a 1:500 dilution, and incubate for 30 minutes at room temperature.
    Lot #_____ Date Reconstituted_____

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

12. Apply the label complex from the Standard Elite Kit, and incubate for 30 minutes at room temperature. (Prepare at least 30 minutes prior to use.)
    Exp. Date_____ New Kit: yes / no

13. Rinse 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 20 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:

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

20. Coverslip