

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

Reagent and Antibody Information

[1X Wash Buffer](#)

[3% Hydrogen Peroxide](#)

[1% BSA Diluent](#)

[DAB Chromogen](#)

[Hematoxylin](#)

Blocking Solution: Dakocytomation Protein Block Serum-Free Ready-To-Use

Dakocytomation Corporation

Carpinteria, CA 93013

www.dako.com

1-800-235-5763

Code No. X0909

Avidin / Biotin Blocking Kit

Vector Laboratories, Inc.

Burlingame, CA 94010

www.vectorlabs.com

1-800-227-6666

Catalog # SP-2001

Primary Antibody: Polyclonal Rabbit Anti-Cytokeratin, Wide Spectrum Screening Antibody

Dakocytomation Corporation

Carpinteria, CA 93013

www.dako.com

1-800-235-5763

Catalog # Z0622

Negative Control Serum: Normal Rabbit Serum

Jackson ImmunoResearch Laboratories, Inc.

West Grove, PA 19390

www.jacksonimmuno.com

1-800-367-5296

Catalog # 011-000-001

Secondary Antibody: Donkey Anti-Rabbit IgG (H+L) Biotin-SP-Conjugated

Jackson ImmunoResearch Laboratories, Inc.

West Grove, PA 19390

www.jacksonimmuno.com

1-800-367-5296

Catalog # 711-065-152

Label Complex: R.T.U. Vectastain Elite ABC Reagent
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog # PK-7100

Staining Procedure

Positive Control Tissue: Gastrointestinal tract or skin epidermis
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. Block with the Dako protein-blocking reagent and incubate for 10 minutes at room temperature.
Lot # _____ Exp. Date _____

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

5. 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.

6. Apply primary antibody at a 1:250 dilution. Incubate for 30 minutes at room temperature.
Lot # _____ Exp. Date _____

For negative control slides, dilute normal rabbit serum so that its protein concentration matches that of the primary antibody (if necessary). Then make a 1:250 dilution. Apply the negative and incubate for 30 minutes at room temperature.

Lot # _____ Date Reconstituted _____

7. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.
8. Apply the donkey anti-rabbit secondary antibody at a 1:500 dilution. Incubate for 30 minutes at room temperature.
Lot # _____ Date Reconstituted _____
9. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.
10. Apply the Vectastain R.T.U. Elite Label and incubate for 30 minutes at room temperature.
Exp. Date _____ New Kit: yes / no
11. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each time.
12. 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
13. Rinse the slides in tap water 3 minutes.
14. Counterstain with hematoxylin for 20 seconds.
15. Rinse the slides in tap water until water is clear.
16. Gently agitate slides in 1X wash buffer until tissues turn blue.
17. 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

18. Coverslip

Updated 03/12/10