

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

Reagents:

[1X Automation Buffer](#)

[3% Hydrogen peroxide](#)

[0.05% Pronase](#)

[Antibody Diluent](#)

[DAB Chromagen](#)

[Hematoxylin](#)

Antibody Information:

Primary antibody: EPOS Vimentin Clone 3B4

Dako Corporation

Carpinteria, CA 93013

Catalog #U7034

Negative antibody: EPOS Negative control

Dako Corporation

Carpinteria, CA 93013

Catalog #U0951

Pronase Solution

According to manufacturer's instructions

Dako Corporation

Carpinteria, CA 93013

Catalog #S2013

Staining Procedure

-Positive Control Tissue: Podocytes of glomeruli in rat kidney

-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 1X Automation Buffer for 5 minutes each.

3. Perform Heat Induced Epitope Retrieval using 0.05% Pronase

Lot # _____

(Dilute 100ul stock in 1.9 mls 1X Automation Buffer)

Equilibrate slides for 5 minutes in 1X Automation Buffer on IQ Stainer at 37 degrees.

Drain buffer from slides and cover with 0.05% Pronase solution.

Incubate slides at 37 degrees for 5 minutes.

Rinse slides in distilled water.

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

7. Incubate sections using prediluted antibodies for 1 hour.

EPOS Vimentin 3B4 Lot # _____ Exp. Date _____

EPOS Negative Control Lot # _____ Exp. Date _____

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

9. Apply liquid Dako DAB Chromagen for 6 minutes in the dark.

(Add 1 drop of DAB per ml of substrate)

Lot # _____ Exp date _____

10. Rinse in tap water 3 minutes.

11. Counterstain with Modified Harris Hematoxylin for 30 seconds.
12. Rinse in tap water until water is clear.
13. Place slides in 1X Automation buffer for 1 minute with gentle agitation to blue slides.
14. Dehydrate through the following solutions.

95% Ethanol	1 change	3 minutes
100% EtOH	3 changes	3 minutes
Xylene	2 changes	5 minutes

15. Coverslip

updated 3/8/04