

Detection of CD24 in Formalin-Fixed, Paraffin-Embedded Mouse Tissue

Reagent and Antibody Information

[1X Wash Buffer](#)

[3% Hydrogen Peroxide](#)

[1% BSA Diluent](#)

[1X Citrate Buffer](#)

[DAB Chromogen](#)

[Hematoxylin](#)

Staining Kit: ImmPRESS Anti-Rat Ig, Mouse adsorbed (peroxidase) Polymer Detection Kit

Vector Laboratories, Inc.

Burlingame, CA 94010

www.vectorlabs.com

1-800-227-6666

Catalog # MP-7444-15

Note: This kit contains a pre-dilute blocking serum and polymer reagent.

Primary Antibody: Rat Anti-Mouse CD24 Monoclonal Antibody

BD Biosciences

San Jose, CA 95131

1-877-232-8995

www.bdbiosciences.com

Catalog # 557436

Lot # 88733

Negative Control Serum: Purified Rat IgG2b Isotype Control Serum

BD Biosciences

San Jose, CA 95131

www.bdbiosciences.com

1-855-236-2772

Catalog # 559478

Staining Procedure

Positive Control Tissue: Fetal liver
Stain Localization: Cell membrane

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 NxGen Decloaking Chamber™
Add 500 ml of distilled water to the pan inside the decloaker. All three of the decloaker's containers must be filled. Any containers without samples should have 250 ml of distilled water. The samples need to be in a container with a full rack of slides and about 200 ml of **1X citrate buffer**. (Insert blank slides into any empty slots in the rack to ensure even heating of slides.)
Decloak the slides for 15 minutes at 110°C. *Maximum Pressure* _____
Remove pan top and cool for 10 minutes. *Temperature Before Cooling Slides* _____
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.
6. Block with the 2.5% normal goat serum from the ImmPRESS kit for 20 minutes at room temperature.
Lot # _____ Exp. Date _____

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

7. Apply primary antibody at a 1:3500 dilution. Incubate for 1 hour at room temperature.
Lot # _____ Exp Date _____

For negative control slides, dilute rat IgG2b control serum so that it's IgG2b protein concentration matches that of the primary antibody (if necessary). Then make a 1:3500 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 _____

8. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.
9. Apply the anti-rat polymer from the ImmPRESS kit, and incubate for 30 minutes at room temperature.
Lot # _____ Exp. Date _____

10. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.
11. 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
12. Rinse the slides in tap water 3 minutes.
13. Counterstain with hematoxylin for 20 seconds.
14. Rinse the slides in tap water until water is clear.
15. Gently agitate slides in 1X wash buffer until the tissues turn blue.
16. 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

17. Coverslip

Updated 12/04/13