

Detection of F4/80 in Formalin-Fixed, Paraffin-Embedded Mouse Tissue

Reagent and Antibody Information

[1X Wash Buffer](#)

[3% Hydrogen Peroxide](#)

[1% BSA Diluent](#)

[1X EDTA](#)

[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 F4/80 Purified Antibody (BM8)

BioLegend

San Diego, CA 92121

www.biolegend.com

1-877-246-5343

Catalog # 123102

Lot # B137300

Negative Control Serum: Purified Rat IgG2a, κ Isotype Control Antibody

BioLegend

San Diego, CA 92121

www.biolegend.com

1-877-246-5343

Catalog # 400502

Staining Procedure

Positive Control Tissue: Spleen - macrophages

Stain Localization: Cytoplasmic and 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. Heat-Induced Epitope Retrieval Using The Decloaker

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 EDTA**. (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 the 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.

3. Rinse slides in 2 changes of 1X wash buffer for 5 minutes each.
4. Quench endogenous peroxidase by placing slides in 3% hydrogen peroxide for 15 minutes.
5. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.
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 SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.
ONLY WIPE EXCESS BLOCK.

7. Apply primary antibody at a 1:500 dilution. Incubate for one hour at room temperature.
Lot# _____ Exp. Date _____

For negative control slides, apply BioLegend rat IgG2a isotype control serum at a 1:500 dilution. Incubate for one hour at room temperature.

Lot# _____ Exp. Date _____

8. Rinse 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 slides in 2 changes of 1X wash buffer for 5 minutes each.
11. Apply the DAB chromogen for 6 minutes in the dark.
(Add 1 drop of DAB per ml of substrate)
Lot# _____ Exp. Date _____ New Kit: yes / no
12. Rinse in tap water 3 minutes.
13. Counterstain with hematoxylin for 20 seconds.
14. Rinse in tap water until water is clear.
15. Agitate slides in 1X wash buffer for 20 seconds.
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 11/15/16