

Monoclonal Antibody to Macrophage F4/80 antigen - Purified

Alternate names:	Cell surface glycoprotein EMR1, EMR1 hormone receptor, Emr1, Gpf480
Catalog No.:	BM4008S
Quantity:	0.25 mg
Concentration:	1.0 mg/ml
Background:	F4/80 antigen is a 160 kD glycoprotein expressed by most murine macrophages. Expression of F4/80 is heterogeneous and is reported to vary during macrophage maturation and activation. The F4/80 antigen is expressed on a wide range of mature tissue macrophages including Kupffer cells, Langerhans, microglia, macrophages located in the gut lamina propria, peritoneal cavity, lung, thymus, bone marrow stroma and macrophages in the red pulp of the spleen. F4/80 expression has also been reported on a subpopulation of dendritic cells but is absent from macrophages located in T cell areas of the spleen and lymphnode. The ligands and biological functions of the F4/80 antigen have not yet been determined but recent studies suggest a role for F4/80 in the generation of efferent CD8+ve regulatory T cells.
Uniprot ID:	Q61549
NCBI:	NP_034260
GeneID:	13733
Host / Isotype:	Rat / IgG2b
Clone:	Cl:A3-1
Immunogen:	Thioglycollate stimulated peritoneal macrophages from C57/BL mice. Spleen cells from immunised HOB2 rats were fused with cells of the mouse NS1 myeloma cell line.
Format:	State: Liquid purified IgG fraction Purification: Affinity Chromatography on Protein G Buffer System: PBS, pH 7.4 Preservatives: 0.09% Sodium Azide
Applications:	RIA. Western Blot. Immunoprecipitation. Immunofluorescence. Immunoelectron Microscopy. Flow Cytometry: Use 10 µl of 1/50-1/100 diluted antibody to label 10 ⁶ cells in 100 µl. Immunohistochemistry on Frozen and Paraffin Embedded and Resin Sections. This product requires pre-treatment of paraffin sections (Proteinase K is recommended for tissues fixed for less than 24 hours. Citrate buffer pH 6.0 is recommended for tissues fixed for more than

24 hours).

Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Specificity:

This antibody recognizes the F4/80 antigen, a member of the EGF-TM7 family of proteins which shares 68% overall amino acid identity with Human EMR1.

Clone *Cl:A31* has been reported to modulate cytokine levels released in response to *Listeria monocytogenes* (Ref.5). We recommend the use of BM4008LE for this purpose.

Species Reactivity: **Tested:** Mouse.

Storage:

Store undiluted at 2-8°C for one month or (in aliquots) at -20°C for longer.

Avoid repeated freezing and thawing.

Shelf life: one year from despatch.

Product Citations: **Purchased from Acris:**

1. Kakehashi H, Nishioku T, Tsukuba T, Kadowaki T, Nakamura S, Yamamoto K. Differential regulation of the nature and functions of dendritic cells and macrophages by cathepsin E. *J Immunol.* 2007 Nov 1;179(9):5728-37. PubMed PMID: 17947645.

2. Kimura H, Miyashita H, Suzuki Y, Kobayashi M, Watanabe K, Sonoda H, et al. Distinctive localization and opposed roles of vasohibin-1 and vasohibin-2 in the regulation of angiogenesis. *Blood.* 2009 May 7;113(19):4810-8. doi: 10.1182/blood-2008-07-170316. Epub 2009 Feb 9. PubMed PMID: 19204325.

3. Cuzi S, Bosnar M, Kramarić MD, Ferencić Z, Marković D, Glojnaric I, et al. Claudin-3 and Clara cell 10 kDa protein as early signals of cigarette smoke-induced epithelial injury along alveolar ducts. *Toxicol Pathol.* 2012 Dec;40(8):1169-87. doi: 10.1177/0192623312448937. Epub 2012 Jun 1. PubMed PMID: 22659244.

Originator or purchased from resellers:

1. Nakao S, Zandi S, Faez S, Kohno R, Hafezi-Moghadam A. Discontinuous LYVE-1 expression in corneal limbal lymphatics: dual function as microvalves and immunological hot spots. *FASEB J.* 2012 Feb;26(2):808-17. doi: 10.1096/fj.11-183897. Epub 2011 Nov 16. PubMed PMID: 22090317.

2. Anghelina M, Krishnan P, Moldovan L, Moldovan NI. Monocytes/macrophages cooperate with progenitor cells during neovascularization and tissue repair: conversion of cell columns into fibrovascular bundles. *Am J Pathol.* 2006 Feb;168(2):529-41. PubMed PMID: 16436667.

General Readings:

1. Austyn JM, Gordon S. F4/80, a monoclonal antibody directed specifically against the mouse macrophage. *Eur J Immunol.* 1981 Oct;11(10):805-15. PubMed PMID: 7308288.

2. Hume DA, Perry VH, Gordon S. The mononuclear phagocyte system of the mouse defined by immunohistochemical localisation of antigen F4/80: macrophages associated with epithelia. *Anat Rec.* 1984 Nov;210(3):503-12. PubMed PMID: 6524692.

3. Lee SH, Starkey PM, Gordon S. Quantitative analysis of total macrophage content in adult mouse tissues. *Immunochemical studies with monoclonal antibody F4/80. J Exp Med.* 1985 Mar 1;161(3):475-89. PubMed PMID: 3973536.

4. Gordon S, Lawson L, Rabinowitz S, Crocker PR, Morris L, Perry VH. Antigen markers of macrophage differentiation in murine tissues. *Curr Top Microbiol Immunol.* 1992;181:1-37. PubMed PMID: 1424778.

5. Warschkau H, Kiderlen AF. A monoclonal antibody directed against the murine macrophage surface molecule F4/80 modulates natural immune response to *Listeria monocytogenes*. *J Immunol.* 1999 Sep 15;163(6):3409-16. PubMed PMID: 10477612.

6. Lin HH, Faunce DE, Stacey M, Terajewicz A, Nakamura T, Zhang-Hoover J, et al. The macrophage F4/80 receptor is required for the induction of antigen-specific efferent regulatory T cells in peripheral tolerance. *J Exp Med.* 2005 May 16;201(10):1615-25. Epub 2005 May 9. PubMed PMID: 15883173.

7. Chan RJ, Leedy MB, Munugalavadla V, Voorhorst CS, Li Y, Yu M, et al. Human somatic

- PTPN11 mutations induce hematopoietic-cell hypersensitivity to granulocyte-macrophage colony-stimulating factor. *Blood*. 2005 May 1;105(9):3737-42. Epub 2005 Jan 11. PubMed PMID: 15644411.
8. Pizza FX, Peterson JM, Baas JH, Koh TJ. Neutrophils contribute to muscle injury and impair its resolution after lengthening contractions in mice. *J Physiol*. 2005 Feb 1;562(Pt 3):899-913. Epub 2004 Nov 18. PubMed PMID: 15550464.
 9. Metwali A, Blum AM, Elliott DE, Setiawan T, Weinstock JV. Cutting edge: hemokinin has substance P-like function and expression in inflammation. *J Immunol*. 2004 Jun 1;172(11):6528-32. PubMed PMID: 15153465.
 10. Moore KJ, Andersson LP, Ingalls RR, Monks BG, Li R, Arnaout MA, et al. Divergent response to LPS and bacteria in CD14-deficient murine macrophages. *J Immunol*. 2000 Oct 15;165(8):4272-80. PubMed PMID: 11035061.
 11. Hemmi H, Idoyaga J, Suda K, Suda N, Kennedy K, Noda M, et al. A new triggering receptor expressed on myeloid cells (Trem) family member, Trem-like 4, binds to dead cells and is a DNAX activation protein 12-linked marker for subsets of mouse macrophages and dendritic cells. *J Immunol*. 2009 Feb 1;182(3):1278-86. PubMed PMID: 19155473.
 12. Seitz O, Schürmann C, Hermes N, Müller E, Pfeilschifter J, Frank S, et al. Wound healing in mice with high-fat diet- or ob gene-induced diabetes-obesity syndromes: a comparative study. *Exp Diabetes Res*. 2010;2010:476969. doi: 10.1155/2010/476969. Epub 2011 Jan 20. PubMed PMID: 21318183.
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 19. Bonde AK, Tischler V, Kumar S, Soltermann A, Schwendener RA. Intratumoral macrophages contribute to epithelial-mesenchymal transition in solid tumors. *BMC Cancer*. 2012 Jan 24;12:35. doi: 10.1186/1471-2407-12-35. PubMed PMID: 22273460.
 20. Choi KM, Kashyap PC, Dutta N, Stoltz GJ, Ordog T, Shea Donohue T, et al. CD206-positive M2 macrophages that express heme oxygenase-1 protect against diabetic gastroparesis in mice. *Gastroenterology*. 2010 Jun;138(7):2399-409. doi: 10.1053/j.gastro.2010.02.014. Epub 2010 Feb 20. PubMed PMID: 20178793.
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Protocols:

1. Enzyme pre-treatment using Proteinase K (Recommended for tissues fixed for 24 hours in neutral buffered formalin, NBF):

Reagents

A. TE buffer (50 mM Tris base, 1 mM EDTA, pH 8.0)

Tris Base, 6.10 g

EDTA, 0.37 g

Distilled water, 1000 ml

Mix to dissolve. Adjust pH to 8.0 using concentrated HCl (10 M HCl). Store at room temperature.

B. Proteinase K stock solution (20x, 400 μ g/ml in TE buffer, pH 8.0)

Proteinase K, 4 mg

TE buffer, pH 8.0, (Reagent A) 10 ml

Mix well. Store in aliquots at -20°C.

C. Proteinase K working solution (1x, 20 μ g/ml in TE buffer, pH 8.0)

Proteinase K stock solution (20x), (Reagent B) 1 ml

TE Buffer, pH 8.0, (Reagent A) 19 ml

Mix well. Discard working solution after use.

Method

1. Dewax paraffin sections and rehydrate using preferred procedure.
2. Cover sections completely with Proteinase K working solution and incubate for 3 minutes at RT.
3. Rinse sections with Phosphate Buffered Saline (PBS).
4. Proceed with serum blocking and preferred staining protocol.

2. Heat-mediated antigen retrieval using citrate buffer, pH 6.0 (Recommended for tissues fixed for 7 days or more in neutral buffered formalin, NBF):

Reagent

Citrate buffer (10 mM citric acid, pH 6.0)

Citric acid (anhydrous), 1.92 g

Distilled water, 1000 ml

Mix to dissolve. Adjust pH to 6.0 with 1 M NaOH (be sure to mix well). Store this solution at RT for 3 months, or at 4°C for longer usage.

Method

1. Dewax paraffin sections and rehydrate using preferred protocol.
2. Pre-heat sodium citrate buffer in a staining vessel to 95-100°C.
3. Immerse slides in the citrate buffer and incubate for 10 minutes at 95-100°C. Check the

citrate buffer level, add more if necessary, and then incubate for a further 10 minutes at 95-100°C.

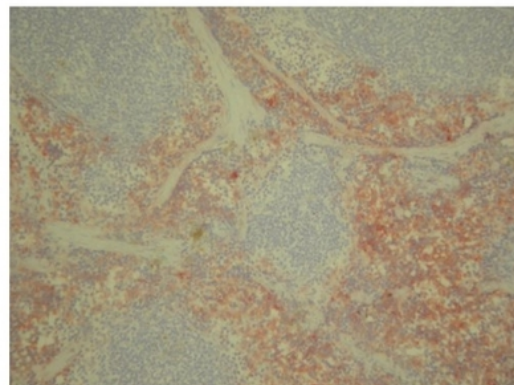
4. Allow sections to cool for 20 minutes.

5. Rinse sections with PBS.

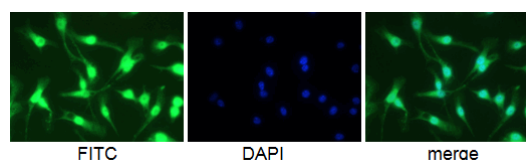
6. Proceed with serum blocking and preferred staining protocol.

Pictures:

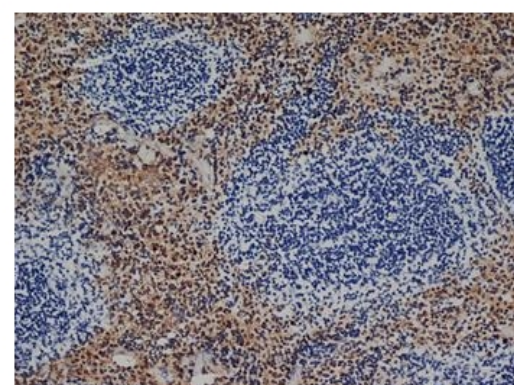
Mouse Spleen Sections stained with Rat anti Mouse F4/80 Antibody Cat.No BM4008



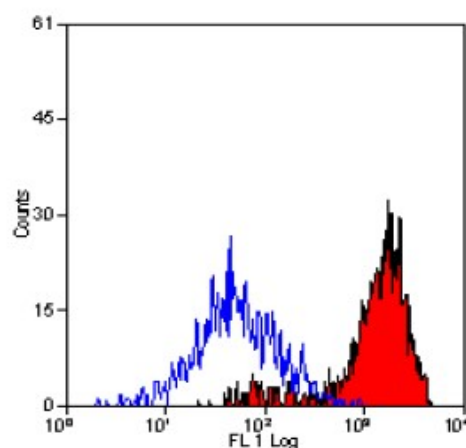
Mouse anti-Macrophage F4/80 antigen antibody Cat.-No BM4008F (5 µg/ml) on Raw 204.4 cells. Cells were fixed in 1% PFA, permeabilized in 0.25% Triton X 100 in PBS, blocked in 1% BSA in PBS.



Frozen Mouse Spleen stained with Rat anti Mouse F4/80 Antibody Cat.No BM4008 using SP1020HRP as the secondary



Staining of Mouse peritoneal macrophages with Rat Anti Mouse F4/80 Antigen (BM4008).



Mouse anti-Macrophage F4/80 antigen antibody Cat.-No BM4008F on thioglycollate elicited mouse peritoneal macrophages. Purple: BM4008F; Green: Isotype control SM19FS; Percentages reflect % positive after subtraction of negative control, using M1 marker.

