

BM4007S**Monoclonal Antibody to Macrophage F4/80 antigen - Aff - Purified**

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| Alternate names: | Cell surface glycoprotein EMR1, EMR1 hormone receptor, Emr1, Gpf480 |
| Quantity: | 0.1 mg |
| Concentration: | 0.2 mg/ml (after reconstitution) |
| Background: | F4/80 protein binds to Macrophages from different sites including the peritoneal cavity, lung, spleen and thymus, to blood monocytes and to macrophages derived from bone marrow precursors in culture. F4/80 is not expressed by lymphocytes or polymorphonuclear cells. |
| Uniprot ID: | Q61549 |
| NCBI: | NP_034260 |
| GeneID: | 13733 |
| Host / Isotype: | Rat / IgG2a |
| Clone: | BM8 |
| Immunogen: | Cultured Macrophages. The antigen/epitope is a 125 kDa extracellular membrane protein sensitive to 2-Mercaptoetanol. |
| Format: | State: Lyophilized purified IgG fraction Purification: Affinity Chromatography Buffer System: Stock Solution contains PBS, pH 7.2 with 5 mg/ml BSA as a stabilizer and 0.01% Kathon as a preservative Reconstitution: Restore by adding 0.5 ml distilled water. |
| Applications: | Immunohistochemistry on Frozen Sections: 0.4 µg/ml (1/500). Immunohistochemistry on Paraffin Sections: 4 µg/ml (1/50). <i>Proteinase K pretreatment</i> for antigen retrieval is recommended. Recommended Positive Control: Mouse spleen. Has been described to work in FACS . Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user. |
| Specificity: | This antibody is useful for the detection of the F4/80 antigen on major subpopulations of resident tissue macrophages. The antigen expression increases upon maturation of macrophage precursors in bone marrow and blood as well as in ontogeny. This clone is the only Macrophage marker that is able to distinguish non-destructive from destructive inflammation processes in the pancreas and has been shown to be a unique histological marker of the progression from peri-insulinitis to beta-cell destruction and diabetes in a mouse diabetes model. Antigen Distribution Tissue Sections: The antigen is detected on tissue fixed macrophages in all organs tested so far (spleen, lymph nodes, thymus, liver, skin). It is also present on |

Langerhans cells in the skin and Kupffer cells in the liver. In complete Freund's adjuvant induced granulomas the antigen is expressed by inflammatory macrophages, but is absent from epitheloid cells.

Isolated Cells: expressed in vitro on over 80% of M-CSF stimulated bone marrow derived macrophages, after a few days of culture. It is absent from granulocytes, lymphocytes and thrombocytes.

Species Reactivity:

Tested: Mouse. Also stains Human Heart Macrophages.

Storage:

Store lyophilized at 2-8°C for 6 months or at -20°C long term.

After reconstitution store the antibody undiluted at 2-8°C for one month or (in aliquots) at -20°C long term.

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. Torzewski M, Shaw PX, Han KR, Shortal B, Lackner KJ, Witztum JL, et al. Reduced in vivo aortic uptake of radiolabeled oxidation-specific antibodies reflects changes in plaque composition consistent with plaque stabilization. *Arterioscler Thromb Vasc Biol.* 2004 Dec;24(12):2307-12. Epub 2004 Nov 4. PubMed PMID: 15528482.
4. Stangl S, Gehrman M, Riegger J, Kuhs K, Riederer I, Sievert W, et al. Targeting membrane heat-shock protein 70 (Hsp70) on tumors by cmHsp70.1 antibody. *Proc Natl Acad Sci U S A.* 2011 Jan 11;108(2):733-8. doi: 10.1073/pnas.1016065108. Epub 2010 Dec 27. PubMed PMID: 21187371.
5. Ilves M, Palomäki J, Vippola M, Lehto M, Savolainen K, Savinko T, et al. Topically applied ZnO nanoparticles suppress allergen induced skin inflammation but induce vigorous IgE production in the atopic dermatitis mouse model. *Part Fibre Toxicol.* 2014 Aug 14;11:38. doi: 10.1186/s12989-014-0038-4. PubMed PMID: 25123235.
6. Kuhs, K. Evaluierung eines tumorspezifischen Antikörpers zur Immuntherapie Hsp70-membranpositiver Tumore im in-vivo Modell. Thesis 2014. <http://mediatum.ub.tum.de/doc/1229327/1229327.pdf>.
7. Lemmermann NA, Krmpotic A, Podlech J, Brizic I, Prager A, Adler H, et al. Non-redundant and redundant roles of cytomegalovirus gH/gL complexes in host organ entry and intra-tissue spread. *PLoS Pathog.* 2015 Feb 6;11(2):e1004640. doi: 10.1371/journal.ppat.1004640. eCollection 2015 Feb. PubMed PMID: 25659098.
8. Braunger BM, Leimbeck SV, Schlecht A, Volz C, Jägle H, Tamm ER. Deletion of Ocular Transforming Growth Factor β Signaling Mimics Essential Characteristics of Diabetic Retinopathy. *Am J Pathol.* 2015 Apr 7. pii: S0002-9440(15)00139-X. doi: 10.1016/j.ajpath.2015.02.007. PubMed PMID: 25857227.

General Readings:

1. Malorny U, Michels E, Sorg C. A monoclonal antibody against an antigen present on mouse macrophages and absent from monocytes. *Cell Tissue Res.* 1986;243(2):421-8. PubMed PMID: 3948241.
2. Kraal G, Rep M, Janse M. Macrophages in T and B cell compartments and other tissue macrophages recognized by monoclonal antibody MOMA-2. An immunohistochemical study. *Scand J Immunol.* 1987 Dec;26(6):653-61. PubMed PMID: 3321409.
3. Leenen PJ, de Bruijn MF, Voerman JS, Campbell PA, van Ewijk W. Markers of mouse macrophage development detected by monoclonal antibodies. *J Immunol Methods.* 1994 Sep 14;174(1-2):5-19. PubMed PMID: 8083537.
4. Smit MJ, Duursma AM, Koudstaal J, Hardonk MJ, Bouma JM. Infection of mice with lactate dehydrogenase-elevating virus destroys the subpopulation of Kupffer cells involved in receptor-mediated endocytosis of lactate dehydrogenase and other enzymes. *Hepatology.* 1990 Nov;12(5):1192-9. PubMed PMID: 2172137.
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Protocols:**Protocol with Frozen, ice-cold Acetone-Fixed Sections:**

(The whole procedure is performed at room temperature)

1. Wash in PBS.
2. Block endogenous peroxidase.
3. Wash in PBS.
4. Block with 10% normal goat serum in PBS for 30min. in a humid chamber.
5. Incubate with primary antibody (dilution see datasheet) for 1h in a humid chamber.
6. Wash in PBS.
7. Incubate with secondary antibody (peroxidase-conjugated goat anti rat IgG (H+L) minimal-cross reaction to mouse) for 1h in a humid chamber.
8. Wash in PBS.
9. Incubate with AEC substrate (3-amino-9-ethylcarbazol) for 12min.
10. Wash in PBS.
11. Counterstain with Mayer's hemalum.

Protocol with Formalin-Fixed, Paraffin-Embedded Sections:

(The whole procedure is performed at room temperature)

1. Deparaffinize and rehydrate tissue section.
2. Incubate the tissue section with proteinase K for 7min.
3. Wash in distilled water.
4. Block endogenous peroxidase.
5. Wash in PBS.
6. Block with 10% normal goat serum in PBS for 30min. in a humid chamber.
7. Incubate with primary antibody (dilution see datasheet) for 1h in a humid chamber.
8. Wash in PBS.

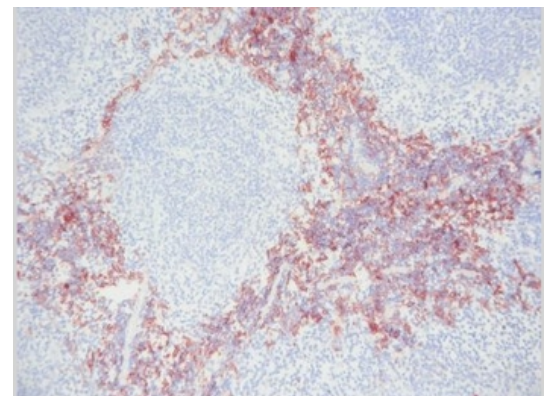
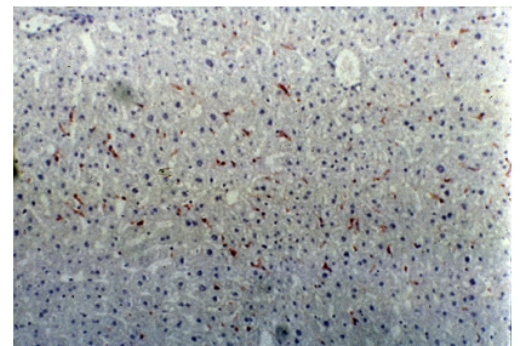
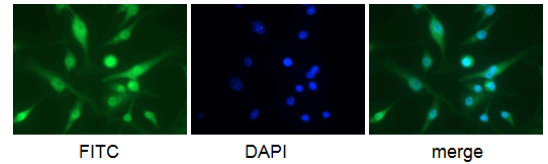
9. Incubate with secondary antibody (peroxidase-conjugated goat anti rat IgG (H+L) minimal-cross reaction to mouse) for 1h in a humid chamber.
10. Wash in PBS.
11. Incubate with AEC substrate (3-amino-9-ethylcarbazol) for 12min.
12. Wash in PBS.
13. Counterstain with Mayer's hemalum.

Pictures:

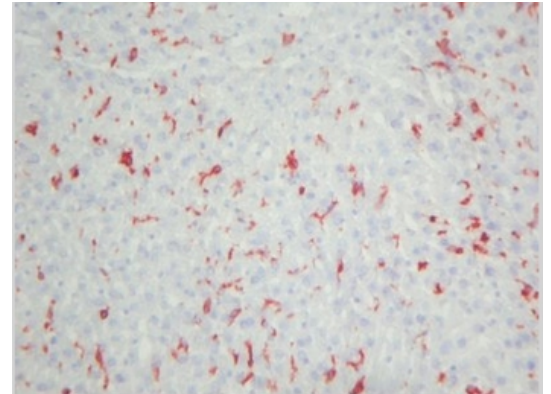
Mouse anti-Macrophage F4/80 antigen antibody Cat.-No. BM4007F (5 µg/ml) on Raw 204.4 cells. Cells were fixed in 1% PFA, permeabilized in 0.25%Triton X 100 in PBS, blocked in 1% BSA in PBS.

BM4007S F4/80 antibody staining of Mouse Liver Section.

BM4007S F4/80 antibody staining of Mouse Spleen Frozen Section.



BM4007S F4/80 Antigen antibody staining of Mouse Liver Frozen Section.



Mouse anti-Macrophage F4/80 antigen antibody Cat.-No. BM4007F on thioglycollate elicited mouse peritoneal macrophages. Purple: BM4007F; Green: Isotype control SM15F; Percentages reflect % positive after subtraction of negative control, using M1 marker.

