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BM4019 Monoclonal Antibody to Ly6c - Purified

Alternate names:	Ly-6C2, Ly6C2, Lymphocyte antigen 6C2, Mouse Macrophage Marker
Quantity:	0.1 mg
Concentration:	0.2 mg/ml
Background:	Ly-6C is a member of the Ly-6 multigene family of type V glycophosphatidylinositol-anchored cell surface proteins. It is expressed on bone marrow cells, monocytes/macrophages, neutrophils, endothelial cells, and T-cell subsets. Mice with the Ly-6.2 allotype (e.g., AKR, C57BL, C57BR, C57L, DBA/2, PL, SJL, SWR, 129) have subsets of CD4+Ly-6C+ and CD8+Ly-6C+ cells, while Ly-6.1 strains (e.g., A, BALB/c, CBA, C3H/He, DBA/1, NZB) have only CD8+Ly-6C+ lymphocytes. Ly-6C may play a role in the development and maturation of lymphocytes.
Uniprot ID:	P09568
NCBI:	NP_001092687.1
GeneID:	17067
Host / Isotype:	Rat / IgG2a
Recommended Isotype Controls:	SM15P, SM15PX
Clone:	ER-MP20
Immunogen:	Mouse macrophage cell lines. **Antigen/Epitope:** The antigen is a glutaraldehyde (0.05%) and paraformaldehyde (1%) resistant 14kD surface protein which is very similar to Ly-6C and may be analogous to Human CD59. It is inducible by IFN-alpha, IFN-beta and IFN-gamma.
Format:	**State:** Lyophilized purified Ig fraction **Purification:** Affinity Chromatography **Buffer System:** PBS, pH 7.2 **Preservatives:** 0.05% (v/v) Kathon CG **Stabilizers:** 5 mg/ml BSA **Reconstitution:** Restore with 0.5 ml distilled water.
Applications:	**Immunohistochemistry on Frozen Sections:** 0.25 µg/ml (1/800) - 0.5 µg/ml (1/400). **Immunohistochemistry on Paraffin Sections:** 0.5 µg/ml (1/400) - 1 µg/ml (1/200). Proteinase K pretreatment for antigen retrieval is recommended. ***Suggested 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 Monoclonl antibody ER-MP20 is useful for the detection of macrophage precursor cells in the mid-stage development stage (late CFU-M, monoblasts and monocytes). It is ideally suitable for the detection of monocytes in bone marrow samples by FACS. ER-MP20 also identifies activated macrophages in inflammatory tissues where the simultaneous use of the murine pan-macrophage marker BM8 (anti F4/80 antibody

BM4007) is recommended. ER-MP20 also detects a wide range of endothelial cells.
Antigen Distribution on Isolated cells: In bone marrow cells the antigen is found on monoblasts and late CFU-M cells as well as on monocytes. It is also found on granulocytes and a subpopulation of lymphocytes in the peripheral blood. Granulocytic cells show a dull, and monocytic cells a bright antigen surface expression. Lymphoid cells express the antigen only very weakly. Thus, in the bone marrow three useful FACS windows can be defined for cell sorting purposes.
Antigen Distribution on Tissue Sections: The antigen is found on macrophage precursor subpopulations in the bone marrow and hemopoietic islands of the lymphoid organs, and in the spleen. Endothelial cells of small vessels in various organs also stain positive with ER-MP20. Activated macrophages in inflammatory tissues also express the ER-MP20-related antigen.

Species Reactivity:

Tested: Mouse (Macrophage precursor cells).

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:

Originator or purchased from resellers:

1. de Bruijn MF, Slieker WA, van der Loo JC, Voerman JS, van Ewijk W, Leenen PJ. Distinct mouse bone marrow macrophage precursors identified by differential expression of ER-MP12 and ER-MP20 antigens. Eur J Immunol. 1994 Oct;24(10):2279-84. PubMed PMID: 7925556.

General Readings:

1. de Bruijn MF, van Vianen W, Ploemacher RE, Bakker-Woudenberg IA, Campbell PA, van Ewijk W, et al. Bone marrow cellular composition in Listeria monocytogenes infected mice detected using ER-MP12 and ER-MP20 antibodies: a flow cytometric alternative to differential counting. J Immunol Methods. 1998 Aug 1;217(1-2):27-39. PubMed PMID: 9776572.
2. Chan J, Leenen PJ, Bertoncello I, Nishikawa SI, Hamilton JA. Macrophage lineage cells in inflammation: characterization by colony-stimulating factor-1 (CSF-1) receptor (c-Fms), ER-MP58, and ER-MP20 (Ly-6C) expression. Blood. 1998 Aug 15;92(4):1423-31. PubMed PMID: 9694732.
3. McCormack JM, Leenen PJ, Walker WS. Macrophage progenitors from mouse bone marrow and spleen differ in their expression of the Ly-6C differentiation antigen. J Immunol. 1993 Dec 1;151(11):6389-98. PubMed PMID: 8245473.
4. Leenen PJ, Melis M, Slieker WA, Van Ewijk W. Murine macrophage precursor characterization. II. Monoclonal antibodies against macrophage precursor antigens. Eur J Immunol. 1990 Jan;20(1):27-34. PubMed PMID: 2407538.
5. Leenen PJ, Slieker WA, Melis M, Van Ewijk W. Murine macrophage precursor characterization. I. Production, phenotype and differentiation of macrophage precursor hybrids. Eur J Immunol. 1990 Jan;20(1):15-25. PubMed PMID: 1968390.
6. Wijffels JF, Hendrickx RJ, Steenbergen JJ, Eestermans IL, Beelen RH. Milky spots in the mouse omentum may play an important role in the origin of peritoneal macrophages. Res Immunol. 1992 May;143(4):401-9. PubMed PMID: 1518954.
7. Leenen PJ, Kroos MJ, Melis M, Slieker WA, van Ewijk W, van Eijk HG. Differential

inhibition of macrophage proliferation by anti-transferrin receptor antibody ER-MP21: correlation to macrophage differentiation stage. Exp Cell Res. 1990 Jul;189(1):55-63. PubMed PMID: 2189739.

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.

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 7 min.
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.

Pictures:

Figure 1. Immunohistochemistry on Mouse Liver Sections using BM4019 Monoclonal antibody (ER-MP20)

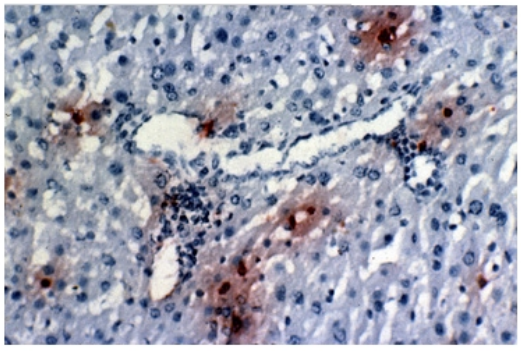

Figure 2. Immunohistochemistry on Mouse spleen Frozen Sections using BM4019 Monoclonal antibody (ER-MP20)