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BM4025 Monoclonal Antibody to MRP8/14 (S100A8/A9) - Purified

Alternate names: CAGA, CAGB, CFAG, CFAG, Calgranulin A/B, Calprotectin, L1 Protein, MRP-14, MRP-8, P14, P8

Quantity: 0.1 mg

Concentration: 0.2 mg/ml (after reconstitution)

Background: MRP8 and MRP14 are members of the S100 family of proteins containing 2 EF hand calcium binding motifs. S100 proteins are localized in the cytoplasm and/or nucleus of a wide range of cells, and involved in the regulation of a number of cellular processes such as cell cycle progression and differentiation. S100 genes include at least 13 members which are located as a cluster on chromosome 1q21.
The antigen is produced by the heterocomplex formation of MRP8 (S100A8 or Calgranulin A) and MRP14 (S100A9 or Calgranulin B), two calcium binding proteins of the S 100 protein family.

Host / Isotype: Mouse / IgG1

Recommended Isotype Controls: SM10P (for use in human samples), AM03095PU-N

Clone: 27E10

Immunogen: Cultured Human monocytes.
Remarks: The antigen is MRP8/14 (Calprotectin), the epitope involves parts of both subunits MRP8 and MRP14.

Format:
State: Lyophilized purifed IgG fraction from cell culture supernatant
Purification: Affinity Chromatography on Protein G
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 (= 0.2 mg/ml Stock Solution).

Applications:
ELISA.
Immunohistochemistry on Frozen Sections: 0.25 µg/ml (1/800).
Immunohistochemistry on Paraffin Sections: 1 µg/ml (1/200). Digestion with Trypsin or Proteinase K pre-treatment for antigen retrieval is recommended.
Recommended Positive Control: Human tonsil.
Has been described to work in **FACS** and **Dot blots.**
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Specificity: This antibody is ideally suited for the detection of early inflammatory macrophages and thus for the classification of acute stage inflammation in tissue sections and in smears, the characterization of tumorous tissues and the in vitro monitoring of peripheral blood cell cultures.
Clone *27E10* is unique in that it recognizes an epitope on the MRP8/14 heterocomplex that is not exposed on the individual subunits MRP8 or MRP14.
The antibody reacts with Human subpopulations of macrophages, monocytes and

granulocytes; peripheral blood monocytes carry the antigen extra- and intracellularly, neutrophils only intracellularly.

Antigen Distribution

Isolated Cells: Monocytes carry the antigen both on the surface and intracellularly, granulocytes exhibit it only intracellularly. Up to 80% of monocytes in early cultures (24-48h) are positive. No reaction has been seen with lymphocytes or platelets.

Tissue Sections: The antigen is found in macrophages in the red pulp of the spleen and liver. It is strongly expressed in macrophages from acute inflamed tissues (peridontitis, contact eczema, urticaria, erythrodermia) where some endothelial and epidermal cells may also express this antigen. It is normally absent on resident mononuclear phagocytes in healthy tissues (skin, gut, thymus).

Species Reactivity:

Tested: Human. The antibody also stains a subpopulation of macrophages of Rhesus Monkey and Bovine tissues. It **does not** react with Swine tissues.

Add. Information:

This antibody was produced serum-free, without fetal calf serum.

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. Altwegg LA, Neidhart M, Hersberger M, Müller S, Eberli FR, Corti R, et al. Myeloid-related protein 8/14 complex is released by monocytes and granulocytes at the site of coronary occlusion: a novel, early, and sensitive marker of acute coronary syndromes. Eur Heart J. 2007 Apr;28(8):941-8. Epub 2007 Mar 26. PubMed PMID: 17387139.

FITC conjugated antibody is cited in:

2. Ghavami S, Rashedi I, Dattilo BM, Eshraghi M, Chazin WJ, Hashemi M, et al. S100A8/A9 at low concentration promotes tumor cell growth via RAGE ligation and MAP kinase-dependent pathway. J Leukoc Biol. 2008 Jun;83(6):1484-92. doi: 10.1189/jlb.0607397. Epub 2008 Mar 13. PubMed PMID: 18339893.

General Readings:

1. Zwadlo G, Schlegel R, Sorg C. A monoclonal antibody to a subset of human monocytes found only in the peripheral blood and inflammatory tissues. J Immunol. 1986 Jul 15;137(2):512-8. PubMed PMID: 3722815.
2. Steinhoff G, Wonigeit K, Sorg C, Behrend M, Mues B, Pichlmayr R. Patterns of macrophage immigration and differentiation in human liver grafts. Transplant Proc. 1989 Feb;21(1 Pt 1):398-400. PubMed PMID: 2650159.
3. Johne B, Fagerhol MK, Lyberg T, Prydz H, Brandtzaeg P, Naess-Andresen CF, et al. Functional and clinical aspects of the myelomonocyte protein calprotectin. Mol Pathol. 1997 Jun;50(3):113-23. PubMed PMID: 9292145.
4. Bröcker EB, Zwadlo G, Holzmann B, Macher E, Sorg C. Inflammatory cell infiltrates in human melanoma at different stages of tumor progression. Int J Cancer. 1988 Apr 15;41(4):562-7. PubMed PMID: 3128489.
5. Frühbeis B, Zwadlo G, Bröcker EB, Schulze Osthoff K, Hagemeier HH, Topoll H, et al. Immunolocalization of an angiogenic factor (HAF) in normal, inflammatory and tumor tissues. Int J Cancer. 1988 Aug 15;42(2):207-12. PubMed PMID: 3403066.
6. Ringler DJ, Walsh DG, MacKey JJ, Hunt RD, King NW. Immunophenotypic

characterization of mononuclear phagocytes and dendritic cells in lymphoid organs of the rhesus monkey. Clin Immunol Immunopathol. 1988 Dec;49(3):349-64. PubMed PMID: 2461268.
7. Roessner A, Herrera A, Höning HJ, Vollmer E, Zwadlo G, Schürmann R, et al. Identification of macrophages and smooth muscle cells with monoclonal antibodies in the human atherosclerotic plaque. Virchows Arch A Pathol Anat Histopathol. 1987;412(2):169-74. PubMed PMID: 3122417.
8. Bhardwaj RS, Zotz C, Zwadlo-Klarwasser G, Roth J, Goebeler M, Mahnke K, et al. The calcium-binding proteins MRP8 and MRP14 form a membrane-associated heterodimer in a subset of monocytes/macrophages present in acute but absent in chronic inflammatory lesions. Eur J Immunol. 1992 Jul;22(7):1891-7. PubMed PMID: 1378023.
9. Burkhardt K, Bösnecker A, Hillebrand G, Hofmann GO, Schneeberger H, Burmeister G, et al. MRP8/14-positive macrophages as early acute cellular rejection markers, and soluble MRP8/14 and increased expression of adhesion molecules following renal transplantation. Transplant Proc. 1995 Feb;27(1):890-1. PubMed PMID: 7533437.
10. Kiefer R, Kieseier BC, Brück W, Hartung HP, Toyka KV. Macrophage differentiation antigens in acute and chronic autoimmune polyneuropathies. Brain. 1998 Mar;121 (Pt 3):469-79. PubMed PMID: 9549523.

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 mouse IgG+IgM (H+L) minimal-cross reaction to human) 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

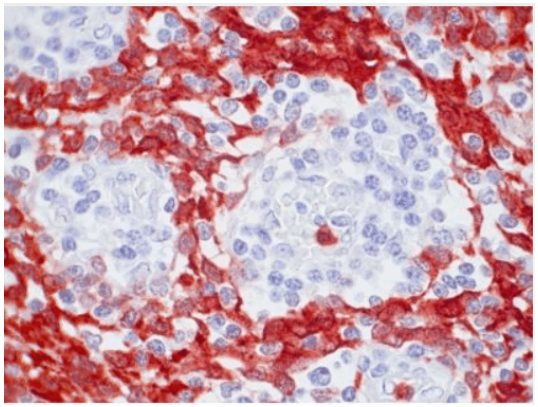
Protocol with formalin-fixed, paraffin-embedded sections:

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 mouse IgG+IgM (H+L) minimal-cross reaction to human) for 1h in a humid chamber
10. Wash in PBS

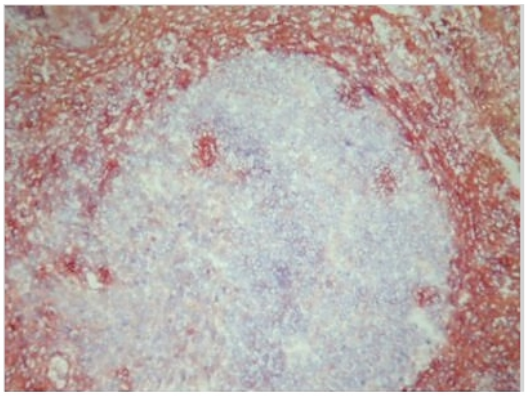
11. Incubate with AEC substrate (3-amino-9-ethylcarbazol) for 12min.
12. Wash in PBS
13. Counterstain with Mayer's hemalum

Pictures:

Human Tonsil Paraffin Section stained with S100A8/9 Antibody Cat.-No BM4025 (Clone 27E10).



Human Tonsil Frozen Section stained with S100A8/9 Antibody Cat.-No BM4025 (Clone 27E10).



Human Tonsil Frozen Section stained with S100A8/9 Antibody Cat.-No BM4025 (Clone 27E10).