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Product datasheet for BM4008LE

Adgre1 Rat Monoclonal Antibody [Clone ID: Cl:A3-1]

Product data:

Product Type:	Primary Antibodies
Clone Name:	Cl:A3-1
Applications:	FC, FN, IF, IHC, IP, R, WB
Recommended Dilution:	**Western Blot.** **Immunoprecipitation.** **Radioimmunoassays.** **Functional Assays.** **Flow Cytometry:** Use 10 µl of 1/100-1/200 diluted antibody to label 10^6 cells in 100 µl. **Immunohistochemistry on Resin Sections.** **Immunohistochemistry on Frozen Sections.** **Immunohistochemistry on Paraffin Sections:** Requires pre-treatment prior to staining. 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.
Reactivity:	Mouse
Host:	Rat
Isotype:	IgG2b
Clonality:	Monoclonal
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.
Specificity:	This antibody recognises the F4/80 antigen. Clone *Cl:A3-1* has been reported to modulate cytokine levels released in response to *Listeria monocytogenes*.
Formulation:	PBS, pH 7.4 State: Low Endotoxin State: Liquid purified IgG fraction Stabilizer: None Preservative: None
Concentration:	lot specific
Purification:	Affinity Chromatography on Protein G

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Conjugation:	Unconjugated
Storage:	Store the antibody undiluted at -20°C. Avoid repeated freezing and thawing.
Stability:	Shelf life: one year from despatch.
Gene Name:	adhesion G protein-coupled receptor E1
Database Link:	Entrez Gene 13733 Mouse Q61549
Background:	F4/80 is a 160 kD cell surface glycoprotein that is a member of the EGF-TM7 family of proteins which shares 68% overall amino acid identity with human EGF module-containing mucin-like hormone receptor 1 (EMR1). 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 lymph node. 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.
Synonyms:	Emr1, Gpf480
Note:	**Endotoxin Level:** < 0.01 Eu/µg

Protocol: **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 µg/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 µg/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

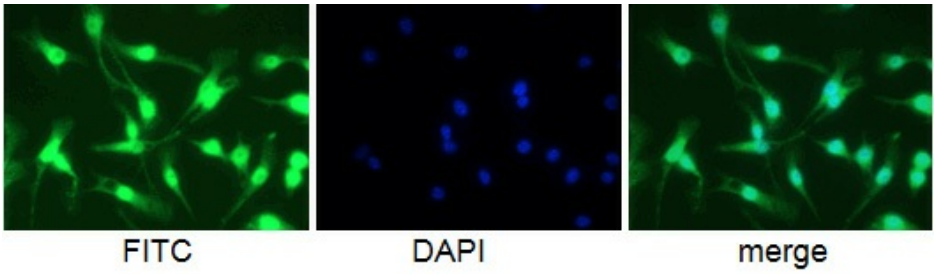
Citrate buffer (10 mM citric acid, pH 6.0)

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.

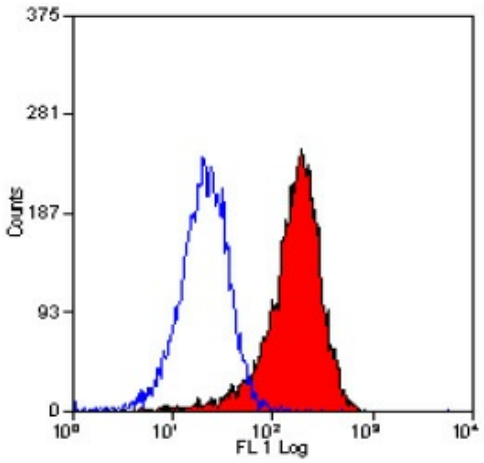
Method

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.

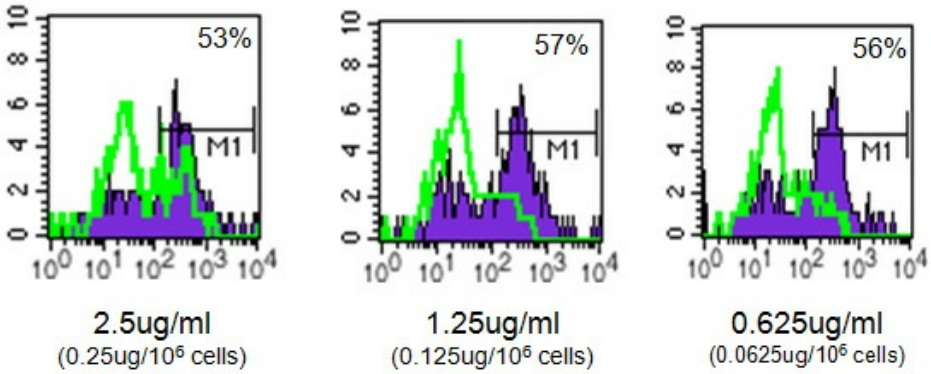
Product images:



Mouse anti-Macrophage F4/80 antigen antibody (5 ug/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.



Staining of J774 cells with Rat Anti Mouse F4/80 Antibody -Low Endotoxin



Mouse anti-Macrophage F4/80 antigen antibody on thioglycollate elicited mouse peritoneal macrophages. Purple: ; Green: Isotype control SM19FS; Percentages reflect % positive after subtraction of negative control, using M1 marker.