OriGene Technologies, Inc.
9620 Medical Center Drive, Ste 200
Rockville, MD 20850, US
Phone: +1-888-267-4436
https://www.origene.com
techsupport@origene.com
EU: info-de@origene.com
CN: techsupport@origene.cn

Product datasheet for AM31834FC-N

C3ar1 Mouse Monoclonal Antibody [Clone ID: 74]

Product data:

Product Type:	Primary Antibodies
Clone Name:	74
Applications:	FC, IHC
Recommended Dilution:	Flow Cytometry. Immunohistochemistry on frozen sections.
Reactivity:	Rat
Host:	Mouse
Isotype:	IgG1
Clonality:	Monoclonal
Immunogen:	RBL-2H3 transfectants expressing rat C3aR Donor: BALB/c Fusion Partner: X63-Ag8.653
Specificity:	This rat C3a receptor antibody detects a recombinant peptide corresponding to amino acids 161-321 of the large extracellular loop structure, which shares 34% homology with mouse and human. Deduced amino acid sequences of human/rat C3aR is 58.5% and 90.7% with rat/mouse.
Formulation:	PBS containing 0.02% sodium azide (NaN3) as preservative and EIA grade BSA was added as a stabilizing protein to bring the total protein concentration to 4-5 mg/ml. Label: FITC State: Liquid purified Ig fraction Label: Fluorescein isothiocyanate isomer 1
Concentration:	lot specific
Purification:	Affinity chromatography on Protein G
Conjugation:	FITC
Storage:	Store the antibody undiluted at 2-8°C for one month or (in aliquots) at -20°C for longer. Avoid repeated freezing and thawing.
Stability:	Shelf life: one year from despatch.
Gene Name:	complement component 3a receptor 1

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Database Link: Entrez Gene 84007 Rat
O55197

Background: C3aR binds to anaphylotoxin C3a which, among other things, is involved in mast cell degranulation and recruitment of immune cells to the site of inflammation. Activation of the complement cascade produces various fragments, including C3a and C5a.
C3aR is characterized by seven transmembrane domains including a large extracellular loop which is coupled to a Gi protein. C3aR expression is detected on glial cells, neurons and cells belonging to the mononuclear phagocyte system. C3aR expression was shown to moderately increase after LPS stimulation.

Synonyms: C3a-R, C3AR, C3AR1, AZ3B, C3R1, HNFAG09

Note:

Protocol: **FLOW CYTOMETRY ANALYSIS:**

Method:

1. Prepare a cell suspension in media A. For cell preparations, deplete the red blood cell population with Lympholyte®-Rat cell separation medium.
2. Wash 2 times.
3. Resuspend the cells to a concentration 2x10e7 cells/ml in media A. Add 50 µl of this suspension to each tube (each tube will then contain 1x10e6 cells, representing 1 test).
4. To each tube, add 0.5µg of this antibody.
5. Vortex the tubes to ensure thorough mixing of antibody and cells.
6. Incubate the tubes for 30 minutes at 4°C.
7. Wash 2 times at 4°C.
8. Add 100 µl of secondary antibody (FITC Goat anti-mouse IgG) at 1/500 dilution.
9. Incubate the tubes at 4°C for 30-60 minutes.

(It is recommended that the tubes are protected from light since most fluorochromes are light sensitive).

10. Wash 2 times at 4°C in media B.
11. Resuspend the cell pellet in 50 µl ice cold media B.
12. Transfer to suitable tubes for flow cytometric analysis containing 15 µl of propidium iodide at 0.5 mg/ml in PBS. This stains dead cells by intercalating in DNA.

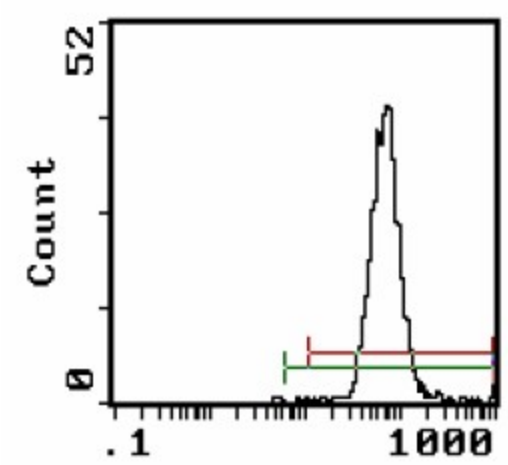
Media:

A. Phosphate buffered saline (pH 7.2) + 5% normal serum of host species + sodium azide (100 µl of 2M sodium azide in 100 mls).
B. Phosphate buffered saline (pH 7.2) + 0.5% Bovine serum albumin + sodium azide (100 µl of 2M sodium azide in 100 mls).

Results:

Tissue Distribution by Flow Cytometry Analysis:
Rat Strain: Wistar
Cell Concentration: 1x10e6 cells per test
Antibody Concentration Used: 2.0µg /106 cells
Isotypic Control: FITC Mouse IgG1
Cell Source:
Peritoneal Macrophages 97.2%
Bone Marrow 81.5%

Product images:



Cell Source: Peritoneal Macrophages Percentage of cells stained above control: 97.2%