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TECHNICALLY Speaking

Place your order with CEDARLANE® or your local distributor.

Please contact CEDARLANE® for lot specific information.

FITC Anti-Rat C3a Receptor (C3aR) Monoclonal Antibody

CL031F
LOT: 3131

DESCRIPTION:
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.

Cedarlane's 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.
This clone is suitable for use in flow cytometry and frozen tissue sections.

PRESENTATION:
100 g FITC labelled Ig buffered in PBS, 0.02% NaN_3 and EIA grade BSA was added as a stabilizing protein to bring the total protein concentration to 4-5 mg/ml.

STORAGE/STABILITY:
Store at + 4°C. For long term storage, aliquot and freeze unused portion at -20°C in volumes appropriate for single usage. Avoid freeze/thaw cycles and prolonged exposure to light.

Continued Overleaf…

For more information or to place an order please contact...

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website: www.cedarlanelabs.com • e-mail: info@cedarlanelabs.com

SPECIFICATIONS:

Clone: 74 (formerly rC3aRZ1)

Hybridoma Production:

Immunization: Immunogen: RBL-2H3 transfectants expressing rat C3aR
Donor: BALB/c

Fusion Partner: X63-Ag8.653

Specificity: Rat C3aR

Ig Class: Mouse IgG_1

Format: FITC conjugated Ig buffered in PBS, 0.02% NaN_3 and EIA grade BSA as a stabilizing protein to bring total protein concentration to 4-5 mg/ml. (Purified from ascitic fluid via Protein G Chromatography)

Antibody Concentration: 0.1 mg/ml

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 (CL5040).
2. Wash 2 times.
3. Resuspend the cells to a concentration $2x10^7$ cells/ml in media A. Add 50 µl of this suspension to each tube (each tube will then contain $1x10^6$ cells, representing 1 test).
4. To each tube, add 2.0µg ***** of **CL031F.**
5. Vortex the tubes to ensure thorough mixing of antibody and cells.
6. Incubate the tubes for 30 minutes at 4°C.
   (It is recommended that the tubes are protected from light, since most fluorochromes are light sensitive.)
7. Wash 2 times at 4°C.
8. Resuspend the cell pellet in 50 µl ice cold media B.
9. 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:

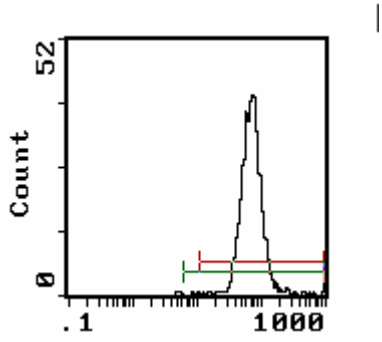
Rat Strain: Wistar

Cell Concentration : $1x10^6$ cells per test

Antibody Concentration Used: 2.0µg /10^6 cells

Isotypic Control: FITC Mouse IgG_1 (CLCMG101)

Cell Source	Percentage of cells stained above control:
Peritoneal Macrophages	97.2%
Bone Marrow	81.5%



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

N.B. Appropriate control samples should always be included in any labelling studies.
*** For optimal results in various applications, it is recommended that each investigator determine dilutions appropriate for individual use.**

REFERENCES:

1) Luhmann, A.E. *et al*. Immunohistochemical analysis of human and rat C3a receptor expression by the use of monoclonal antibodies. *Molecular Immunology* (Abstracts), 2001., **38**:108.
2) Fukuoka, Y., *et al*. Cloning and characterization of rat C3a receptor: differential expression of rat C3a and C5a receptors by LPS stimulation. *Biochem. And Biophys. Res. Comm*. 1998, **242**: 663.
3) Francis, K., *et al*. Complement C3a receptors in the pituitary gland: a novel pathway by which an innate immune molecule releases hormones involved in the control of inflammation. *The FASEB journal*. 2003, **17**: 2266.
4) Benard, M., *et al*. Characterization of C3a and C5a receptors in rat cerebellar granule neurons during maturation. *J. Biol. Chem*. 2004, **279** (42): 43487.

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