



Monoclonal Anti-human/mouse PBEF Antibody

ORDERING INFORMATION

Catalog Number: MAB4044

Clone: 362616

Lot Number: YML01

Size: 100 µg

Formulation: 0.2 µm filtered solution in PBS with 5% trehalose

Storage: -20° C

Reconstitution: sterile PBS

Specificity: human or mouse PBEF

Immunogen: NS0-derived rmPBEF

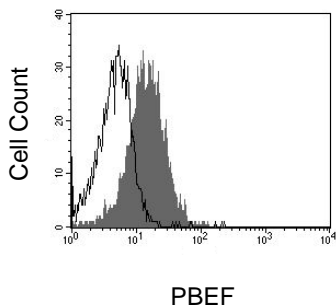
Ig class: rat IgG_{2A}

Recommended Applications:

Western blot
Flow cytometry

Other Application:

Direct ELISA



Murine 3T3-L1 cells were stained with anti-PBEF (R&D Systems, Cat. # MAB4044, filled histogram) or isotype control (R&D Systems, Cat. # MAB0061, open histogram) followed by APC-conjugated anti-rat antibody (R&D Systems, Cat. # F0113).

Preparation

This antibody was produced from a hybridoma resulting from the fusion of a mouse myeloma with B cells obtained from a rat immunized with purified, NS0-derived, recombinant mouse pre-b-cell colony-enhancing factor 1 (rmPBEF; aa 1 - 491: Accession # AAH18358). The IgG fraction of the tissue culture supernatant was purified by Protein G affinity chromatography. PBEF, also called Nampt or visfatin, is a ubiquitous 52 kDa nicotinamide phosphoribosyltransferase. It is the rate-limiting component in the biosynthesis of NAD⁺, and functions in the cytoplasm to regulate energy metabolism during stress responses and immune activation. Although it lacks a signal sequence, PBEF appears to be secreted by visceral adipose and functions as a noncompetitive insulin mimetic. Mouse PBEF shows 96% and > 99% aa identity with human and rat PBEF, respectively.

Formulation

Lyophilized from a 0.2 µm filtered solution in phosphate-buffered saline (PBS) with 5% trehalose.

Reconstitution

Reconstitute with sterile PBS. If 0.2 mL of PBS is used, the antibody concentration will be 500 µg/mL.

Storage

Lyophilized samples are stable for twelve months from date of receipt when stored at -20° C to -70° C. Upon reconstitution, the antibody can be stored at 2° - 8° C for 1 month without detectable loss of activity. Reconstituted antibody can also be aliquotted and stored frozen at -20° C to -70° C in a **manual defrost freezer** for six months without detectable loss of activity. **Avoid repeated freeze-thaw cycles.**

Specificity

This antibody was selected for its ability to detect rmPBEF in direct ELISAs and Western blots. In these formats, this antibody shows 100% cross-reactivity with rhPBEF.

Applications

Western blot - This antibody can be used at 1 - 2 µg/mL with the appropriate secondary reagents to detect human or mouse PBEF. Using a colorimetric detection system, the detection limit for rmPBEF is approximately 10 ng/lane under non-reducing and reducing conditions. Chemiluminescent detection with WesternGlo™ Chemiluminescent Detection Substrate (R&D Systems, Catalog # AR004) will increase sensitivity by 5 to 50 fold.

Flow cytometry - This antibody was validated for flow cytometry using murine 3T3-L1 cells. For intracellular staining to detect PBEF, cells must first be fixed and permeabilized using 4% paraformaldehyde and 0.1% saponin in PBS. Dilute this antibody to 25 µg/mL and add 10 µL of the diluted solution to 1 - 5 x 10⁵ cells in a total reaction volume not exceeding 200 µL. The binding of unlabeled antibodies may be visualized by adding a secondary developing reagent such as anti-rat IgG conjugated to a fluorochrome.

Direct ELISA - This antibody can be used at 0.5 - 1.0 µg/mL with the appropriate secondary reagents to detect human or mouse PBEF. The detection limit for rmPBEF is approximately 4 ng/well.

Optimal dilutions should be determined by each laboratory for each application.

FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.

R&D Systems, Inc.
1-800-343-7475

11/12/07