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TECHNICALLY Speaking

Place your order with CEDARLANE® or your local distributor.

Please contact CEDARLANE® for lot specific information.

Purified Anti-Guinea Pig DAF (Decay-Accelerating Factor) Monoclonal Antibody

CL063AP
LOT:6321

DESCRIPTION:

Cedarlane's Purified Anti-Guinea Pig DAF (decay-accelerating factor) monoclonal antibody (Clone: 44) detects guinea pig DAF; one of several membrane proteins that prevent host cells from homologous complement attack. There are multiple isoforms in guinea pig detected at 55, 70 and 88kDa in SDS page, and their expression does not appear to be tissue or cell-type specific[4]. Transmembrane, GPI-anchored and secreted isoforms are found in most tissues, but their relative amounts differ. For example, the GPI-anchored forms are predominantly found in intestine and testis, while the transmembrane forms are predominantly expressed in lung, bladder, ovary and fetal lung[2]. In contrast, the transmembrane form of mouse DAF is predominantly found in mouse testis[2].

This antibody is suitable for use in flow cytometry, Western Blotting and immunohistochemistry (frozen sections).

PRESENTATION:

250 µg purified IgG buffered in PBS and 0.02% NaN_3.

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.

Continued Overleaf…

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

CEDARLANE® LABORATORIES LIMITED

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SPECIFICATIONS:

Clone: MCA44

Hybridoma Production:

Immunization:
Immunogen: Neu-GPE (neuraminidase-treated guinea pig erythrocytes)
Donor: BALB/c spleen

Fusion Partner: P3U1

Specificity: Guinea Pig DAF

Ig Class: Mouse IgG1

Format: Purified IgG buffered in PBS and 0.02% NaN_3. (Purified from ascitic fluid via Protein G Chromatography).

Antibody Concentration: 1.0 mg/ml

FLOW CYTOMETRY ANALYSIS:

1. Prepare cell suspension in Media A. For cell preparations, deplete the red blood cell population with ammonium chloride (NH_4Cl).
2. Wash 2 times.
3. Resuspend cells to $1x10^6$ cells in approximately 50 µl Media A in a microcentrifuge tube (i.e. 50 µl of cells resuspended to $2x10^7$ cells/ml).
   (the contents of 1 tube represent 1 test).
4. To each tube add 0.2 µg* of **CL063AP** per 10^6 cells.
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 **CLCC30204** (PE Goat anti-mouse IgG (H+L)) at 1:20 dilution.
9. Incubate 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 phosphate buffered saline. (This stains dead cells by intercalating 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).

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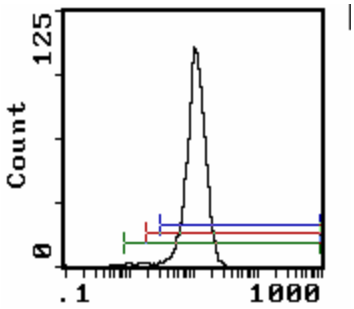
Results:

Tissue Distribution by Flow Cytometry Analysis:

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

Antibody Concentration Used: $0.2\mu g/10^6$ cells
Isotypic Control: Mouse IgG1 (CLCMG100)

Cell Source	Percentage of cells stained above control:
Bone Marrow	93.1%
Blood	98.9%
Spleen	2.9%



Cell Source: Blood
Percentage of cells stained above control: 98.9%

N.B. Appropriate control samples should always be included in any labeling studies.

*** For optimal results in various applications, it is recommended that each investigator determine dilutions appropriate for individual use.**

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2. Wang, G., Nonaka, M., He, C., Okada, N., Nakashima, I., Okada, H. 1998. Functional Differences Among Multiple Isoforms of Guinea Pig Decay-Accelerating Factor. The Journal of Immunology. 160: 3014-3022.

3. Okada, N., Tanaka, H., Takizawa, H., Okada, H. 1995. A Monoclonal Antibody That Blocks the Complement Regulatory Activity of Guinea Pig Erythrocytes and Characterization of the Antigen Involved as Guinea Pig Decay-Accelerating Factor. The Journal of Immunology. 154: 6103-6111.

4. Nonaka, M., Miwa, T., Okada, N., Nonaka, M., Okada, H. 1995. Multiple Isoforms of Guinea Pig Decay-Accelerating Factor (DAF) Generated by Alternative Splicing. The Journal of Immunology. 155: 3037-3048.

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