

ORDERING INFORMATION

Catalog Number: MAB5376

Clone: 583905

Lot Number: CEBQ01

Size: 100 µg

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

Storage: -20° C

Reconstitution: sterile PBS

Specificity: mouse CD55

Immunogen: NS0-derived rmCD55

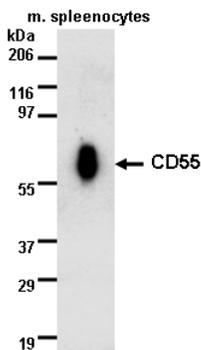
Ig class: rat IgG_{2A}

Recommended Applications:

Western blot
Flow cytometry

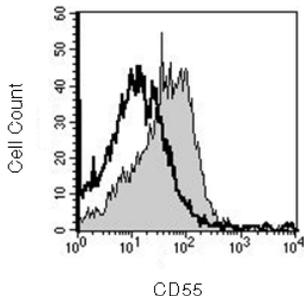
Other Application:

Direct ELISA



Detection of mouse CD55 with MAB5376.

Lysates were prepared from murine spleenocytes in non-reducing sample buffer, resolved by SDS-PAGE (30 µg total protein/lane), and transferred to an Immobilon-P membrane. Use of this antibody under reducing conditions is not recommended. The blot was developed with 2 µg/mL MAB5376 overnight at 4° C and chemiluminescent detection substrate.



Splenocytes were stained with anti-mouse CD55 (Catalog # MAB5376, filled histogram) or isotype control (Catalog # MAB006, open histogram) followed by PE-conjugated anti-rat antibody (Catalog # F0105B).

FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.

Background

CD55 (Decay-accelerating factor/DAF) is a glycoprotein member of the RCA family of molecules. It is found on blood cells, epithelium and endothelium, and serves both as a receptor for CD97, and a negative regulator of the C3 convertases, C4b2a and C3bBb. Mature mouse CD55 is the product of two genes that arose by duplication. There is a 55 - 60 kDa, 356 amino acid (aa), GPI-linked form that is ubiquitously expressed. This molecule contains four SUSHI domains (aa 35 - 285), a Ser/Thr-rich region (aa 288 - 362), and a GPI-anchor at Gly362. There is also a 50 kDa, 379 aa, type I transmembrane form that is testis-associated. It shows the same domain architecture and is 93% aa identical to the GPI-form. At least four GPI gene isoforms exist. They diverge after Ile285 and show deletions and substitutions. Over aa 35 - 359, mouse CD55 is 66% and 50% aa identical to rat and human CD55, respectively.

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 CD55 (rmCD55; aa 1 - 359; Accession # Q61475). The IgG fraction of the tissue culture supernatant was purified by Protein G affinity chromatography.

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 detects rmCD55 in direct ELISAs and Western blots. In direct ELISAs, this antibody shows no cross-reactivity with rhCD55 or rmCD97.

Applications

Western blot - This antibody can be used at 2 µg/mL with the appropriate secondary reagents to detect mouse CD55 in cell or tissue extracts. Refer to <http://www.RnDSystems.com/go/WBCeLLysates> for detailed procedures for preparing lysates and Western blotting. The specific buffers are listed below.

Blotting Buffer

25 mM Tris, pH 7.4
0.15 M NaCl
0.05% Tween® 20

Blocking Solution

5% nonfat dry milk
in Blotting Buffer

Antibody Solution

5% nonfat dry milk
in Blotting Buffer

Flow cytometry - This antibody was tested for flow cytometry using spleenocytes (Harris, C.L. *et al.* (1999) *Biochem J.* **341**:821). Dilute this antibody to 25 µg/mL and add 10 µL of the diluted solution to 1 - 2.5 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. Between steps, wash cells in Flow Cytometry Staining Buffer (R&D Systems, Catalog # FC001) or equivalent.

Direct ELISA - This antibody can be used at 0.5 - 1.0 µg/mL with the appropriate secondary reagents to detect mouse CD55. The detection limit for rmCD55 is approximately 2 ng/well.

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