Monoclonal Anti-human/mouse VAMP-1/VAMP-2 Antibody

ORDERING INFORMATION

Catalog Number: MAB5958

Clone: 540524

Lot Number: CCWM01

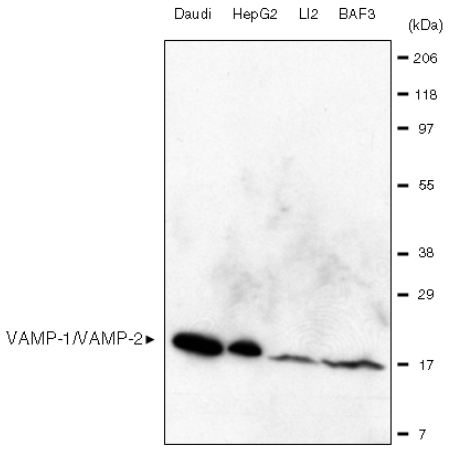
Size: 100 µg

Specificity: human/mouse VAMP-1

Immunogen: *E. coli*-derived rhVAMP-1

Ig class: mouse IgG_1

Recommended Applications:
Western blot
Immunohistochemistry



Detection of VAMP-1 with MAB5958.
30 µg of whole cell extracts from exponentially growing human Daudi and HepG2 and mouse LI2 and BAF3 cells were prepared, resolved by SDS-PAGE, and transferred to a PVDF membrane. The membrane was immunoblotted with 0.2 µg/mL mouse anti-VAMP-1 antibody. *Protocols for Immunoblotting.*

Background
VAMP-1 (vesicle-associated membrane protein 1; also synaptobrevin-1/SYB1) is an 18 kDa member of the synaptobrevin family of proteins. It is expressed in neurons, neutrophils and skeletal muscle cells, and participates in vesicle fusion with the plasma membrane. Human VAMP-1 is a type IV transmembrane protein that contains an N-terminal cytoplasmic region (aa 1 - 96) and a 22 aa transmembrane domain (aa 97 - 118). There is one coiled-coil region between aa 33 - 93. Multiple splice variants are known that show two, three, four and 81 aa substitutions for the C-terminal five amino acids. One three aa variant creates a mitochondrial targeting motif. Over aa 1 - 96, human VAMP-1 is 98% aa identical to mouse VAMP-1.

Preparation
This antibody was produced from a hybridoma resulting from the fusion of a mouse myeloma with B cells obtained from a mouse immunized with purified, *E. coli*-derived recombinant human VAMP-1 (rhVAMP-1; aa 1 - 96; Accession # P23763). 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
The antibody detects endogenous human/mouse VAMP-1 and VAMP-2 on Western blots with an approximate molecular weight of 16 kDa (it does not react with recombinant VAMP family members 5, 7 and 8).

Applications
Western blot - An antibody concentration of 0.2 µg/mL is recommended.

Protocols for Immunoblotting

Blotting Buffer	Blocking Solution	Antibody Solution
25 mM Tris, pH 7.4	5% nonfat dry milk	5% nonfat dry milk
0.15 M NaCl	in Blotting Buffer	in Blotting Buffer
0.1% Tween® 20	Adjust pH to 7.4	Adjust pH to 7.4

1. Transfer the electrophoresed proteins to a PVDF membrane and incubate the membrane for 1 hour at room temperature in Blocking Solution.
2. Incubate the membrane 1 hour room temperature in Antibody Solution containing 0.2 µg/mL mouse anti-human/mouse VAMP-1.
3. Wash the membrane at room temperature for 30 minutes with 3 or more changes of Blotting Buffer.
4. Incubate the membrane at room temperature for 1 hour in Antibody Solution containing a 1:1,000 dilution of HRP-conjugated goat anti-mouse IgG (R&D Systems, Catalog # HAF007).
5. Wash the membrane for 30 minutes with 3 or more changes of Blotting Buffer.
6. Detect with chemiluminescent detection reagent.

Cell lysates for Western blottings - A single plate (150 mm) of exponentially growing cells is washed twice in cold PBS. 1 mL of boiling 1% SDS lysis buffer (1% SDS, 10 mM Tris-HCl, pH 7.4, 1 mM sodium ortho-vanadate) is added to the plate. The plate is then scraped and the lysis is collected, sonicated and quantified. 30 µg of cellular protein is added to an equal amount of 2x SDS loading buffer. Samples are then boiled for 5 minutes and run on a SDS-PAGE gel.

Immunohistochemistry - This antibody was used at a concentration of 25 µg/mL with appropriate secondary reagents to detect VAMP-1/VAMP-2 in paraffin-embedded normal human globus pallidus tissue sections. For chromogenic detection of labeling, the use of R&D Systems Cell and Tissue Staining Kits (CTS Series) is recommended.

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

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