

Affinity-purified Goat Anti-human/mouse WASF3/WAVE3 Antibody

ORDERING INFORMATION

Catalog Number: AF5515

Lot Number: CCCD01

Size: 100 µg

Storage: -20° C

Specificity: human/mouse WASF3/WAVE3

Immunogen: *E. coli*-derived rhWASF3/WAVE3
(aa 1 - 180)

Ig Type: goat IgG

Application: Western blot

Background

WASF3/WAVE3 (Wiscott-Aldrich syndrome protein family Verprolin-homologous protein 3) is a 70 kDa member of the SCAR/WAVE family of proteins. It is expressed in select diverse cell types such as neurons, platelets, and breast epithelium. Upon activation, WASF3/WAVE3 is phosphorylated on Try151/248/337/486 and, in complex with HSPC300, initiates actin polymerization. WASF3/WAVE3 also regulates MMP expression. Human WASF3/WAVE3 is 502 amino acids (aa) in length. It contains one coiled-coil region (aa 57 - 93), three sequential poly-Pro regions (aa 304 - 410) and one VPH domain (aa 440 - 457) that binds to actin and Arp2/3. There is one isoform that shows a 36 aa substitution for aa 181 - 239. Over aa 1 - 180, human WASF3 shares 98% aa identity with mouse WASF3.

Preparation

Goat antibodies were raised against purified, *E. coli*-derived recombinant human WASF3/WAVE3 (rhWASF3/WAVE3; aa 1 - 180; Accession # Q9UPY6). Polyclonal antibody was affinity-purified on a column derivatized with the recombinant protein and further purified by isolating the IgG fraction.

Formulation

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

Reconstitution

Reconstitute in PBS containing 0.02% Na₂S₂O₃.

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 endogenous human/mouse WASF3/WAVE3 in Western blot with an approximate molecular weight of 70 kDa.

Application

Western blot - An antibody concentration of 1.0 µg/mL is recommended.

Protocols for Immunoblotting

Blotting Buffer

25 mM Tris, pH 7.4
0.15 M NaCl
0.1% Tween® 20

Blocking Solution

5% nonfat dry milk
in Blotting Buffer
Adjust pH to 7.4

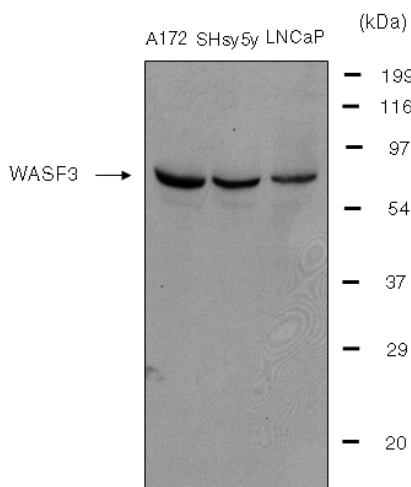
Antibody Solution

5% nonfat dry milk
in Blotting Buffer
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 at room temperature in Antibody Solution containing 1.0 µg/mL goat anti-human/mouse WASF3/WAVE3.
3. Wash the membrane at room temperature for 30 minutes with 3 or more changes of Blotting Buffer. Changing the membrane containers often reduces background.
4. Incubate the membrane at room temperature for 1 hour in Antibody Solution containing a 1:1,000 dilution of HRP-conjugated donkey anti-goat IgG (R&D Systems, Catalog # HAF109).
5. Wash the membrane for 30 minutes with 3 or more changes of Blotting Buffer.
6. Detect with chemiluminescent detection reagents.

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.

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



Detection of WASF3/WAVE3 with AF5515.

30 µg of whole cell extracts from exponentially growing A172, SHsy5y, and LNCaP cells were prepared, resolved by SDS-PAGE, and transferred to a PVDF membrane. The membrane was immunoblotted with 1.0 µg/mL goat anti-WASF3/WAVE3 antibody.