

Product Information

Anti-ASAP1/Centaurin β 4 (ED-20)

Developed in Rabbit, IgG Fraction of Antiserum

Product Number **A 4227**

Product Description

Anti-ASAP1/Centaurin β 4 (ED-20) is developed in rabbit using a synthetic peptide located near the C-terminus of mouse ASAP1 (amino acids 1128-1147), conjugated to KLH as immunogen. The peptide sequence is identical in bovine ASAP1/DEF-1 and mouse ASAP1a and ASAP1b isoforms. Whole antiserum is fractionated and then further purified by ion-exchange chromatography. Anti-ASAP1/Centaurin β 4 recognizes human ASAP1 by immunoblotting (130 kDa, doublet band). Staining of ASAP1 in immunoblotting is specifically inhibited with the ASAP1 immunizing peptide.

ADP-ribosylation factors (ARFs) family of GTP binding proteins are involved in the regulation of membrane trafficking and actin cytoskeleton.¹⁻² ASAP1 (ARF GAP containing SH3, ANK repeat and PH domains 1, also known as DEF-1 or centaurin β 4) is a 130 kDa, ADP-ribosylation factor GTPase activating protein (ARF-GAP).³ ARFs require ARF-GAPs that stimulate their GTPase activity. ASAP1 belongs to a family of related ARF-GAP proteins, consisting of PAP/DEF-2, ACAP1, ACAP2, GIT1/CAT1/APP1, and GIT2/CAT2/PKL, which are involved in the regulation of the actin cytoskeleton and cell migration.⁴

ASAP1 binds ARF1 and ARF5, with only modest activity toward ARF6. It also binds to other known regulators of actin cytoskeleton, such as tyrosine kinase Src, Crk, FAK and phosphatidylinositol-4,5-bisphosphate (PIP2).^{3,5-7} ASAP1 is phosphorylated on tyrosine residues in cells expressing activated Src. ASAP1a and ASAP1b are two variants formed by alternative splicing of the ASAP1 gene. ASAP1 localizes to focal adhesions and cycles with focal adhesion proteins when cells are stimulated to move. Overexpression of ASAP1 alters the morphology of focal adhesions, blocks cell spreading and formation of dorsal ruffles, and prevents the efficient organization of paxillin and FAK in focal adhesions.^{6,7} On the other hand, a mutation disrupting GAP activity of ASAP1 or overexpression of a truncated variant of ASAP1 that fails to bind FAK has a reduced effect on inhibition of

cell spreading. By directly interacting with both ARFs and focal adhesion proteins such as paxillin and FAK, ASAP1 plays an important role in the regulation of focal adhesion assembly and cytoskeletal remodeling.

Reagent

The antibody is provided in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide as a preservative.

Precautions and Disclaimer

Due to the sodium azide content, a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazardous and safe handling practices.

Storage/Stability

Store at -20°C . For extended storage, upon initial thawing, freeze in working aliquots. Do not store in frost-free freezers. Avoid repeated freezing and thawing to prevent denaturing the antibody. Working dilution samples should be discarded if not used within 12 hours. The antibody is stable for at least 6 months when stored appropriately.

Product Profile

A recommended working dilution of 1:2,500 is determined by immunoblotting, using rat brain and mouse brain extracts. For immunofluorescence a minimum working dilution of 1:1,000 is recommended on mouse NIH3T3 fibroblast cells.

Note: In order to obtain best results in different techniques and preparations we recommend determining optimal working concentration by titration test.

References

1. Moss, J., and Vaughan, M., J. Biol. Chem., **270**, 12327-12330 (1995).
2. Donaldson, J.G., and Klausner, R.D., Curr. Opin. Cell Biol., **6**, 527-532 (1994).
3. Brown, M.T., et al., Mol. Cell. Biol., **18**, 7038-7051 (1998).

4. Turner, C.E., et al., Curr. Opin. Cell Biol., **13**, 593-599 (2001).
5. Kam, J.L., et al., J. Biol. Chem., **275**, 9653-9663 (2000).
6. Randazzo, P.A., et al., Proc. Natl. Acad. Sci. USA, **97**, 4011-4016 (2000).
7. Liu, Y., et al., Mol. Biol. Cell, **13**, 2147-2156 (2002).

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