

Certificate of Analysis

Product: Affinity Purified Anti-ASAP1 pY782 (Rabbit)

Code: 600-401-910

Lot #: 16942

Size: 100 µg

Physical State: Liquid (sterile filtered)

Antibody Concentration: 1.09 mg/ml (by UV absorbance at 280 nm)

Buffer: 0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2

Stabilizer: None

Preservative: 0.01% (w/v) Sodium Azide

Storage Conditions: Store vial at -20° C prior to opening. Dilute only prior to immediate use. For extended storage aliquot contents and freeze at -20° C or below. Avoid cycles of freezing and thawing. Expiration date is one (1) year from date of opening.

Background Information: ASAP1 (also known as AMAP1, 130-kDa phosphatidylinositol 4,5-biphosphate-dependent ARF1 GTPase-activating protein, PIP2-dependent ARF1 GAP,ADP-ribosylation factor-directed GTPase-activating protein 1, ARF GTPase-activating protein 1, Development and differentiation-enhancing factor 1, Differentiation-enhancing factor 1, DEF-1) is an Arf-directed GTPase activating protein that is a substrate for the kinases Src and FAK and has been implicated in the regulation of membrane traffic, focal adhesions and invadopodia/podosomes. Phosphorylation of ASAP1 at tyrosine 782 has been found to affect enzymatic and some biological activities, including the function of invadopodia. ASAP1 is expressed in many tissues but is most abundant in the testis, brain, lung and spleen. A heightened expression was seen in the adipose tissue from obese (ob) and diabetic (db) animals. Multiple transcript variants have been reported for this protein.

Application Note(s): This affinity purified antibody has been tested for use in ELISA, immunohistochemistry, IF microscopy and by western blot. Specific conditions for reactivity should be optimized by the end user. Expect a band approximately 130 kDa in size corresponding to phosphorylated ASAP1 protein by western blotting in the appropriate cell lysate or extract. Less than 2.5% reactivity is observed against the non-phosphorylated form of the immunizing peptide. This antibody is phospho specific for pY782 of ASAP1 protein.

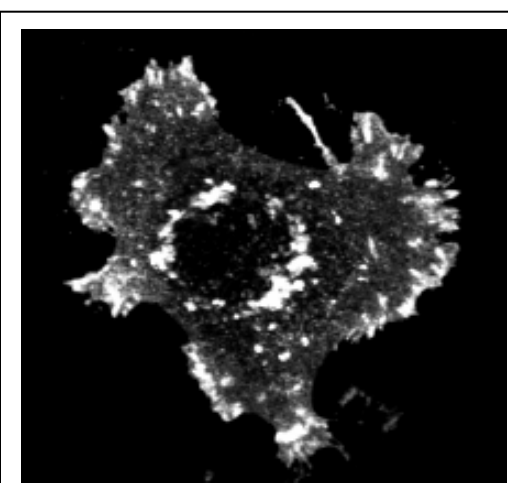


Figure 1. Immunofluorescent microscopy using Rockland's Affinity Purified anti-ASAP1 pY782 antibody shows detection of phosphorylated ASAP1 present in mouse NIH3T3 cells transfected with activated Src. Specific staining is not present when antibody is pre-incubated with the immunizing peptide prior to reaction with cells. *Personal Communication. Paul Randazzo, NIH, CCR, Bethesda, MD.*

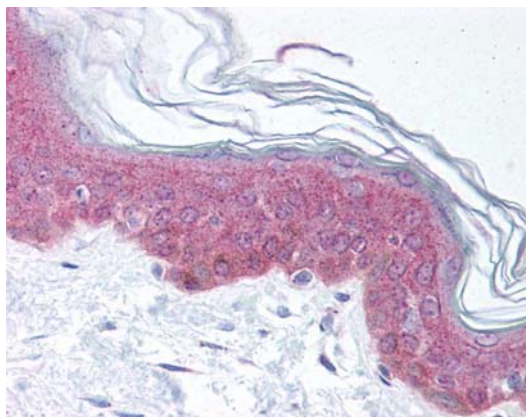


Figure 2. Immunohistochemistry. ROCKLAND's affinity purified anti-ASAP1 pY782 antibody was used at 20 µg/ml to detect signal in a variety of tissues including multi-human, multi-brain and multi-cancer slides. This image shows moderate intracellular positive staining in epidermal keratinocytes in human skin at 40X. Tissue was formalin-fixed and paraffin embedded. The image shows localization of the antibody as the precipitated red signal, with a hematoxylin purple nuclear counterstain. *Personal Communication, Tina Roush, LifeSpanBiosciences, Seattle, WA.*

Recommended Dilutions:	ELISA	1:4,000 - 1:16,000
	WESTERN BLOT	1:500 - 1:2,000
	IMMUNOHISTOCHEMISTRY	20-40 µg/ml
	OTHER APPLICATIONS	User Optimized

Purity and Specificity: This affinity-purified antibody is directed against the phosphorylated form of mouse ASAP1 protein at the pY782 residue. The product was affinity purified from monospecific antiserum by immunoaffinity purification. Antiserum was first purified against the phosphorylated form of the immunizing peptide. The resultant affinity purified antibody was then cross-adsorbed against the non-phosphorylated form of the immunizing peptide. Reactivity occurs against mouse ASAP1 pY782 protein and the antibody is specific for the phosphorylated form of the protein. Reactivity with non-phosphorylated mouse ASAP1 is minimal by ELISA. A BLAST analysis was used to suggest cross reactivity with ASAP1 proteins from human, chicken, bovine, dog, rat and chimpanzee based on 100% homology with the immunizing sequence. Reactivity against homologues from other sources is not known.

Immunogen: This affinity purified antibody was prepared from whole rabbit serum produced by repeated immunizations with a synthetic peptide corresponding to amino acids 777-787 of mouse ASAP1 protein.

Relevant Links: NCBI [NP_034156](#) (see also [AAC98349](#)) Swiss-Prot [Q9QWY8](#)

General References:

- Brown MT, Andrade J, Radhakrishna H, Donaldson JG, Cooper JA, Randazzo PA. (1998) ASAP1, a phospholipid-dependent Arf GTPase-activating protein that associates with and is phosphorylated by Src. *Mol. Cell Bio.* **18**: 7038-7051.
- Onodera Y, Hashimoto S, Hashimoto A, Morishige M, Mazaki Y, Yamada A, Ogawa E, Adachi M, Sakurai T, Manabe T, Wada H, Matsuura N, Sabe H. Expression of AMAP1, an ArfGAP, provides novel targets to inhibit breast cancer invasive activities. *EMBO J.* **24** : 963-973.
- Liu, Y., Yerushalmi, G.M., Grigera, P.R. and Parsons, J.T. (2005) Mislocalization or reduced expression of Arf GTPase-activating protein ASAP1 inhibits cell spreading and migration by influencing Arf1 GTPase cycling. *J. Biol. Chem.* **280** (10), 8884-8892.
- Kruljac-Leticic, A., Moelleken, J., Kallin, A., Wieland, F. and Blaukat, A. (2003) The tyrosine kinase Pyk2 regulates Arf1 activity by phosphorylation and inhibition of the Arf-GTPase-activating protein ASAP1. *J. Biol. Chem.* **278** (32), 29560-29570.
- Liu, Y., Loijens, J.C., Martin, K.H., Karginov, A.V. and Parsons, J.T. (2002) The association of ASAP1, an ADP ribosylation factor-GTPase activating protein, with focal adhesion kinase contributes to the process of focal adhesion assembly. *Mol. Biol. Cell* **13** (6), 2147-2156.
- Furman, C., Short, S.M., Subramanian, R.R., Zetter, B.R. and Roberts, T.M. (2002) DEF-1/ASAP1 is a GTPase-activating protein (GAP) for ARF1 that enhances cell motility through a GAP-dependent mechanism. *J. Biol. Chem.* **277** (10), 7962-7969.
- Yamabhai, M. and Kay, B.K. (1997) Examining the specificity of Src homology 3 domain--ligand interactions with alkaline phosphatase fusion proteins. *Anal. Biochem.* **247** (1), 143-151.

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- [#611-144-122](#) DyLight™680 Conjugated Affinity Purified Anti-RABBIT IgG (H&L) (GOAT) MX10
- [#MB-070](#) Blocking Buffer for Fluorescent Western Blotting
- [#KIA-003](#) **MaxTag™** Anti-RABBIT IgG Kit for Immunoblotting

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