

### Certificate of Analysis

**Product:** Affinity Purified Anti-SUMO Activating Enzyme E1 (SAE1) pS185 [Rabbit]

**Code:** 600-401-B24

**Lot #:** 23460

**Size:** 100 µg

**Physical State:** Liquid (sterile filtered)

**Antibody Concentration:** 0.71 mg/ml (by UV absorbance at 280 nm)

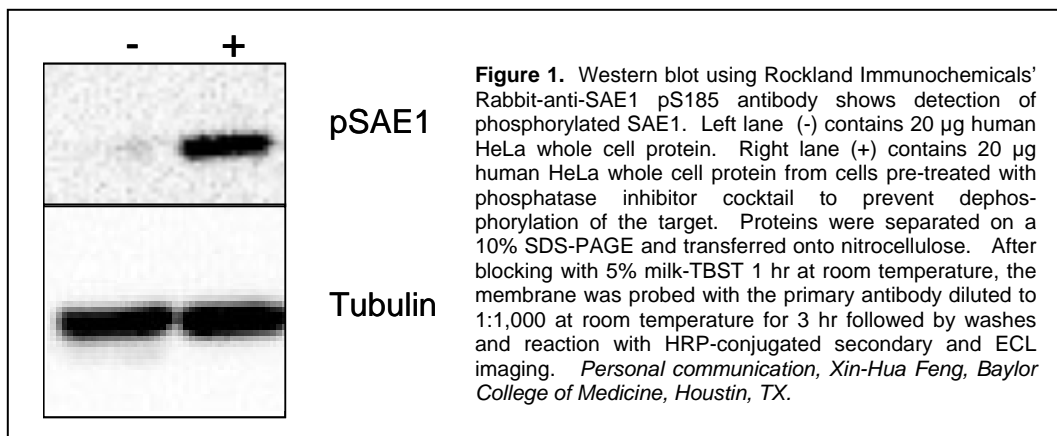
**Buffer:** 0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2

**Stabilizer:** None

**Preservative:** 0.01% (w/v) Sodium Azide

**Storage Conditions:** Store vial at -20° C prior to opening. Aliquot contents and freeze at -20° C or below for extended storage. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4°C as an undiluted liquid. Dilute only prior to immediate use. Expiration date is one (1) year from date of opening.

**Background:** SUMO E1 activating enzyme (also called Ubiquitin-like 1 activating enzyme E1A, UBLE1A, AOS1, SAE1, and SUA1) with SAE2 (also known as UBA2) forms a heterodimeric (SAE1/SAE2) enzyme that activates the ubiquitin-like SUMO proteins (SUMO stands for Small Ubiquitin-like Modifier.) The SAE1 (SUMO Activating Enzyme 1) subunit resembles the N-terminal half of yeast UBA1; the SAE2 (also called Uba2) subunit corresponds to the C-terminal part of yeast UBA1 and contains the active site cysteine. In the SUMO activation step, SAE1/SAE2 uses ATP to adenylate the C-terminal glycine of SUMO-1 (the first of the three different mammalian SUMO proteins) then forms a high-energy thioester bond between the C-terminal glycine and the active site cysteine in SAE2 (Uba2). In the conjugation step, the SUMO moiety is transferred from SAE1/SAE2 to the active site cysteine (Cys 93) of the SUMO conjugating enzyme (SUMO E2, Ubc9) forming a SUMO-E2 thioester complex.



**Application Note(s):** This purified antibody has been tested for use in ELISA and western blot. Specific conditions for reactivity should be optimized by the end user. Expect a band at ~37 kDa in size corresponding to phosphorylated SAE1 protein by western blotting in the appropriate cell lysate or extract. This phospho-specific antibody reacts with human SAE1 pS185 and shows minimal reactivity by ELISA against the non-phosphorylated form of the immunizing peptide.

<b>Recommended Dilutions:</b>	<b>ELISA</b>	1:100,000 - 1:125,000
	<b>WESTERN BLOT</b>	1:750 - 1:1,000
	<b>IF MICROSCOPY</b>	User Optimized
	<b>OTHER APPLICATIONS</b>	User Optimized

**Purity and Specificity:** This purified antibody is directed against human SUMO Activating Enzyme E1 protein. The product was purified from monospecific antiserum by affinity chromatography. This antibody is specific for human SAE1 protein phosphorylated at S185. A BLAST analysis using the sequence of the immunizing peptide was used to suggest that this antibody would react with SUMO Activating Enzyme E1 protein from human (100%), bovine, dog, chimpanzee (96%), mouse (93%), and rat (92%) based on a high degree of sequence homology. Cross reactivity against this protein from other sources has not been determined.

**Relevant Link(s):** Swiss-Prot [Q9UBE0](#) NCBI Link [Q9UBE0](#)

**Immunogen:** This purified antibody was prepared from whole rabbit serum produced by repeated immunizations with a synthetic peptide corresponding to a region surrounding S185 of the human SUMO Activating Enzyme E1 protein.

#### General References:

Wrighton KH, and Feng XH. (2006). Uba2. *AfCS-Nature Molecule Pages*. doi:10.1038/mp.a003681.01

Lois, L.M. and Lima, C.D. (2005) Structures of the SUMO E1 provide mechanistic insights into SUMO activation and E2 recruitment to E1. *EMBO J.* **24** (3), 439-451

Desterro, J.M., Rodriguez, M.S., Kemp, G.D. and Hay, R.T. (1999) Identification of the enzyme required for activation of the small ubiquitin-like protein SUMO-1. *J. Biol. Chem.* **274** (15), 10618-10624.

Gong, L., Li, B., Millas, S. and Yeh, E.T. (1999) Molecular cloning and characterization of human AOS1 and UBA2, components of the sentrin-activating enzyme complex. *FEBS Lett.* **448** (1), 185-189.

**Related Products:** (visit [www.rockland-inc.com](http://www.rockland-inc.com) for a complete listing of Ubiquitin related antibodies)

# <a href="#">200-401-913</a>	IgG fraction of Anti-SUMO Activating Enzyme E1 (SAE1) (Rabbit)
# <a href="#">200-401-431</a>	IgG fraction of Anti-UBIQUITIN (Rabbit)
# <a href="#">200-401-441</a>	IgG fraction of Anti-Human SUMO (Rabbit)
# <a href="#">W09-000-361</a>	A431 Whole Cell Lysate in SDS-PAGE Sample Buffer
# <a href="#">611-703-127</a>	Peroxidase Conjugated Affinity Purified Anti-RABBIT IgG (H&L) (DONKEY) MX10
# <a href="#">611-132-122</a>	IRDye800 Conjugated Affinity Purified Anti-RABBIT IgG (H&L) (GOAT) MX10
# <a href="#">MB-070</a>	Blocking Buffer for Fluorescent Western Blotting
# <a href="#">KIA-003</a>	<b>MaxTag</b> <sup>TM</sup> Anti-RABBIT IgG Kit for Immunoblotting

**Note:** This product is for research use only and is not intended for therapeutic or diagnostic applications. Please contact a technical service representative for more information. All products of animal origin manufactured by Rockland Immunochemicals are derived from starting materials of North American origin. Collection was performed in United States Department of Agriculture (USDA) inspected facilities and all materials have been inspected and certified to be free of disease and suitable for exportation. All properties listed are typical characteristics and are not specifications. All suggestions and data are offered in good faith but without guarantee as conditions and methods of use of our products are beyond our control. All claims must be made within 30 days following the date of delivery. The prospective user must determine the suitability of our materials before adopting them on a commercial scale. Suggested uses of our products are not recommendations to use our products in violation of any patent or as a license under any patent of Rockland Immunochemicals, Inc. If you require a commercial license to use this material and do not have one, then return this material, unopened to: Rockland Inc., P.O. BOX 326, Gilbertsville, Pennsylvania, USA.