SAE1 [GST-tagged] / **SAE2** [6His-tagged]

E1 Activating Enzyme

Alternate Names: SAE1 = Activator of SUMO1, AOS1; SAE2 = Ubiquitin-Like Modifier-Activating Enzyme 2, UBA2

Cat. No.	**61-0005-010**	Quantity:	10 µg
Lot. No.	**30078**	Storage:	-70˚C

FOR RESEARCH USE ONLY NOT FOR USE IN HUMANS

Background

The enzymes of the SUMOylation pathway play a pivotal role in a number of cellular processes including nuclear transport, signal transduction, stress responses and cell cycle progression. The covalent modification of proteins by small ubiquitin-related modifiers (SUMOs) may modulate their stability and subcellular compartmentalisation. Three classes of enzymes are involved in the process of SUMOylation; an activating enzyme (E1), conjugating enzyme (E2) and protein ligases (E3s). SAE1/SAE2 is a SUMO1, 2 and 3 E1 activating enzyme and functions as a heterodimer. Cloning of the human SAE1 and SAE2 genes was first described by Desterro *et al.* (1999). SAE1 and SAE2 share sequence similarity to the N-terminus and C-terminus of ubiquitin E1 activating enzymes respectively (Desterro *et al.* 1999). SAE2 harbours the E1-like active cysteine site while SUMO1 transfer to the E2 conjugating enzyme UBE2I requires both of the SAE subunits (Desterro *et al.* 1999). A crystal structure of the SAE1/SAE2 dimer together with the SUMO1 adenylate has been solved at 2.45 Ångström resolution (Olsen *et al.* 2010). Western blot analysis of cell-cycle synchronised HeLa cells demonstrated increased SAE1 expression in S phase followed by a decrease in G2 phase. Immunofluorescence showed that SAE1 and SAE2 were distributed throughout the nuclei but were excluded from the nucleoli (Azuma *et al.* 2001). A short hairpin RNA (shRNA) screen was carried out

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Physical Characteristics

Species: human

Source: *E. coli*

Quantity: 10 µg

Concentration: 0.5 mg/ml

Formulation: 50 mM HEPES pH 7.5, 150 mM sodium chloride, 2 mM dithiothreitol, 10% glycerol

Molecular Weight: SAE1 = 64.8 kDa
SAE2 = 73.4 kDa

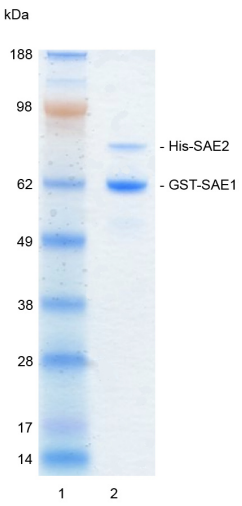
Purity: >98% by InstantBlue™ SDS-PAGE

Stability/Storage: 12 months at -70˚C; aliquot as required

Protein Sequences: Please see page 2

Quality Assurance

Purity:
4-12% gradient SDS-PAGE
InstantBlue™ staining
Lane 1: MW markers
Lane 2: 1 µg SAE1/SAE2



Protein Identification:
Confirmed by mass spectrometry.

E1 Thioester SUMO Loading Assay:
The activity of GST-SAE1/His-SAE2 was validated by loading SUMO1 onto the active cysteine of GST-SAE1/His-SAE2. Incubation of the GST-SAE1/His-SAE2 enzyme in the presence of SUMO1 and ATP at 30˚C was compared at two time points, T_0 and T_{10} minutes. Sensitivity of the SUMO / GST-SAE1/His-SAE2 thioester bond to the reducing agent DTT was confirmed.

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Lot-specific COA version tracker: v1.0.0

SAE1 [GST-tagged] / **SAE2** [6His-tagged]

E1 Activating Enzyme

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Background

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in the presence of aberrant MYC signalling to identify genes that altered the fitness of mammary epithelial cells. In this screen SAE1 and SAE2 were identified as MYC synthetic lethal genes. Upon MYC hyperactivation inactivation of SAE2 led to mitotic catastrophe and cell death and it is thought that SAE2 inactivation could be a therapeutic strategy in MYC driven cancers (Kessler *et al.* 2012).

References:

Azuma Y, Tan SH, Cavenagh MM, Ainsztein AM, Saitoh H, et al. (2001) Expression and regulation of the mammalian SUMO-1 E1 enzyme. *FASEB J* **15**, 1825-1827.

Desterro JM, Rodriguez MS, Kemp GD, Hay RT (1999) Identification of the enzyme required for activation of the small ubiquitin-like protein SUMO-1. *J Biol Chem* **274**, 10618-10624.

Kessler JD, Kahle KT, Sun T, Meerbrey KL, Schlabach MR, et al. (2012) A SUMOylation-dependent transcriptional subprogram is required for Myc-driven tumorigenesis. *Science* **335**, 348-353.

Olsen SK, Capili AD, Lu X, Tan DS, Lima CD (2010) Active site remodelling accompanies thioester bond formation in the SUMO E1. *Nature* **463**, 906-912.

Physical Characteristics

Continued from page 1

SAE1 Protein Sequence:

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEH
LYERDEGDKWRNKKFELGLEFPNLPYYIDGD
VKLTQSMAIIRYIADKHNMLGGCPKERAEISM
LEGAVLDIRYGVSRIAYSKDFETLKVDFL
SKLPEMLKMFEDRLCHKTYLNGDHVTHPD
FMLYDALDVVLYMDPMCLDAFPKLVCFK
KRIEAIPQIDKYLKSSKYIAWPLQGWQATFG
GGDHPPKSDLVPRGS***M***VEKEEAGGGISEEE
AAQYDRQIRLWGLEAQKRLRASRVLLVGLK
GLGAEIAKNLILAGVKGLTMLDHEQVTPEDP
GAQFLIRTGSVGRNRAEASLERAQNLNPMVD
VKVDTEDIEKKPESFFTQFDAVCLTCCSRD
VIVKVDQICHKNSIKFFTGDVFGYHGYTFAN
LGEHEFVEEKTKVAKVSQGVEDGPDTKRAK
LDSSETTMVKKKVVFCPVKEALEVDWSSEKA
KAALKRTTSDYFLLQVLLKFRTDKGRDPSSD
TYEEDSELLLQIRNDVLDSLGISPDLLPED
FVRYCFSEMAPVCAVVGGILAQEIVKALSQRD
PPHNNFFFFDGMKGNGIVECLGPK

Tag **(bold text)**: N-terminal GST
Protease cleavage site: Thrombin (LVPR▼GS)
SAE1 (regular text): Start ***bold italics*** (amino acid residues 1-346)
Accession number: NP_005491.1

SAE2 Protein Sequence:

MPWHHHHHHLEVLFQGP***M***ALSRGLPRELAE
AVAGGRVLVVGAGGIGCELLKNLVLTGF
SHIDLIDLDTIDVSNLNRQFLFQKKHVGR
SKAQVAKESVLQFYPKANIVAYHDSIMNPDYN
VEFFRQFILVMNALDNRAARNHVNRMCLAADV
PLIESGTAGYLGQVTTIKKGVTECYECHP
KPTQRTFPGCTIRNTPSEPIHCIVWAKYLF
NQLFGEEDADQEVSPDRADPEAAWEPTEAEA
RARASNEDGDIKRISTKEWAKSTGYDPVKLFT
KLFKDDIRYLLTMDKLWRKRKPPVPLD
WAEVQSQGEETNASDQQNEPQLGLKDQQVLD
VKSYARLFSKSIETLRVHLAEKGDGAELIWDKD
DPSAMDFVTSAANLRMHIFSMNMKSRFDIKS
MAGNIIPAIATTNAVIAGLIVLEGLKILS
GKIDQCRTIFLNKQPNPRKKLLVPCALDPPNPN
CYVCASKPEVTVRLNVHKVTVLTLQDKIVKEK
FAMVAPDVQIEDGKGTILISSEEGETEANNH
KKLSEFGIRNGSRLQADDFLQDYTLLINILH
SEDLGKDVEFEVVGDAPEKVGPKQAEDAAKSIT
NGSDDGAQPSTSTAQEQDDVLIVDSDEEDSSN
NADVSEEERSRKRKLDEKENLSAKRSRIEQ
KEELDDVIALD

Tag **(bold text)**: N-terminal His
Protease cleavage site: PreScission™ (LEVLFQ▼GP)
SAE2 (regular text): Start ***bold italics*** (amino acid residues 1-640)
Accession number: NP_005490.1

To purify the SAE1 [GST-tagged] / SAE2 [6His-tagged] heterodimer the genes for these two proteins were co-expressed using a prokaryotic expression vector. Nickel resin capture was performed on cell lysate derived from the lysed *E. coli* expressing the two proteins. Glutathione resin capture was then performed on the eluate from the Nickel capture step to capture SAE1 [GST-tagged] / SAE2 [6His-tagged] heterodimer which was then eluted and dialysed into the storage buffer

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Lot-specific COA version tracker: v1.0.0