

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
Lot. No. 30078

Quantity: 10 µg
Storage: -70°C



FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS

CERTIFICATE OF ANALYSIS Page 1 of 2

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 Å 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

Continued on page 2

Physical Characteristics

Species: human

Protein Sequences: Please see page 2

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

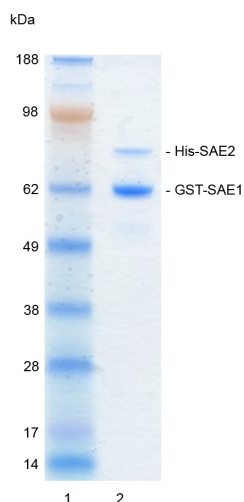
Molecular Weight: SAE1 = 64.8 kDa
SAE2 = 73.4 kDa

Purity: >98% by InstantBlue™ SDS-PAGE

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

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, T₀ and T₁₀ minutes. Sensitivity of the SUMO / GST-SAE1/His-SAE2 thioester bond to the reducing agent DTT was confirmed.



www.ubiquigent.com
Dundee, Scotland, UK

ORDERS / SALES SUPPORT

International: +1-617-245-0003
US Toll-Free: 1-888-4E1E2E3 (1-888-431-3233)
Email: sales.support@ubiquigent.com

UK HQ and TECHNICAL SUPPORT

International: +44 (0) 1382 381147 (9AM-5PM UTC)
US/Canada: +1-617-245-0020 (9AM-5PM UTC)
Email: tech.support@ubiquigent.com

Email services@ubiquigent.com for enquiries regarding compound profiling and/or custom assay development services.

© Ubiquigent 2012. Unless otherwise noted, Ubiquigent, Ubiquigent logo and all other trademarks are the property of Ubiquigent, Ltd.

Limited Terms of Use: For research use only. Not for use in humans or for diagnostics. Not for distribution or resale in any form, modification or derivative OR for use in providing services to a third party (e.g. screening or profiling) without the written permission of Ubiquigent, Ltd.

Lot-specific COA version tracker: v1.0.0

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
Lot. No. 30078

Quantity: 10 µg
Storage: -70°C



FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS

CERTIFICATE OF ANALYSIS Page 2 of 2

Background

Continued from page 1

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
KRIEAIPOIDKYLKSSKYIAWPLQGWQATFG
GGDHPKSDLVPRGSMVEKEEAGGGISEEE
AAQYDRQIRLWGLEAQKRLRASRVLLVGLK
GLGAEIAKNLILAGVKGLTMDHEQVTPEDP
GAQFLIRTSVGRNRAEASLERAQNLNPMVD
VKVDTEIEKKPESFFTFDAVCLTCCSRD
VIVKVDQICHKNSIKFFTGDVFGYHGYTFAN
LGEHEFVEEKTAKVAVSQGVEDGPDTKRAK
LDSSETTMVKKKVVFCPVKEALEVDWSSEKA
KAALKRRTTSDYFLLQVLLKFRDTKGRDPSSD
TYEEDSELLLOIRNDVLDLSLGISPDLLPED
FVRYCFSEMAPVCAVVGILAQEIVKALSQRD
PPHNNFFFDGMKNGIIVECLGPK**

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:

**MPWHHHHHHLEVLFOGPMALSRGLPRELAE
AVAGGRVLVVGAGGIGCELLKNLVLVTGF
SHIDLIDLDTIDVSNLNRQFLFQKKHVGR
SKAQVAKESVLQFYPKANIVAYHDSIMNPDYN
VEFFRQFILVMNALDNRAARNHVNRMCCLADV
PLIESGTAGYLGQVTTIKKGVTECYECP
KPTQRTFPGCTIRNTPSEPIHCIVWAKYLF
NQLFGEEDADQEVSPDRADPEAAWEPTEAEA
RARASNEDGDIKRISTKEWAKSTGYDPVKLFT
KLFKDDIRYLLTMDKLWRKRKPPVPLD
WAEVQSQGEETNASDQQNEPQLGLKDQQVLD
VKSARLFSKSIETLRVHLAEKGDGAELIWDKD
DPSAMDFVTSANLRMHIFSMNMKSRFDIKS
MAGNIIPAIATTNAVIAGLIVLEGLKILS
GKIDQCRTIFLNKQPNRKKLLVPCALDPPNPN
CYVCASKPEVTVRLNVHKVTVLTLQDKIVKEK
FAMVAPDVQIEDGKGTILISSEEGETEANNH
KKLSEFGIRNGSRLQADDFLDQYTLINILH
SEDLGKDVFEVVGDAPEKVGPKQAEDAASIT
NGSDDGAQPSTSTAQEQQDDVLIIVDSDEEDSSN
NADVSEERSRKRKLDEKENLSAKRSRIEQ
KEELDDVIALD**

Tag (**bold text**): N-terminal His
Protease cleavage site: PreScission™ (LEVLFO▼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



www.ubiquigent.com
Dundee, Scotland, UK

ORDERS / SALES SUPPORT

International: +1-617-245-0003
US Toll-Free: 1-888-4E1E2E3 (1-888-431-3233)
Email: sales.support@ubiquigent.com

UK HQ and TECHNICAL SUPPORT

International: +44 (0) 1382 381147 (9AM-5PM UTC)
US/Canada: +1-617-245-0020 (9AM-5PM UTC)
Email: tech.support@ubiquigent.com

Email services@ubiquigent.com for enquiries regarding compound profiling and/or custom assay development services.

© Ubiquigent 2012. Unless otherwise noted, Ubiquigent, Ubiquigent logo and all other trademarks are the property of Ubiquigent, Ltd.

Limited Terms of Use: For research use only. Not for use in humans or for diagnostics. Not for distribution or resale in any form, modification or derivative OR for use in providing services to a third party (e.g. screening or profiling) without the written permission of Ubiquigent, Ltd.

Lot-specific COA version tracker: v1.0.0