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1-866-9 KINASE (54-6273)

Catalog #	Aliquot Size
U208-380G-20	20 µg
U208-380G-50	50 µg

SAE1/UBA2 Complex, Active

Recombinant full-length human proteins expressed in Sf9 cells

Catalog # U208-380G

Lot # D2411-3

Product Description

Recombinant full-length human SAE1 (UBLE1A) and human UBA2 (SAE2) were co-expressed by baculovirus in Sf9 insect cells. SAE1 used an N-terminal his tag, and the gene accession number is NM_005500. UBA2 used an N-terminal GST tag, and the gene accession number is NM_005499.

Gene Aliases

SAE1: UBLE1A; SUA1; HSPC140; AOS1; FLJ3091;
UBA2: SAE2; UBLE1B; SAE2; ARX; HRIHFB2115;

Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 50mM NaCl, 10mM glutathione, 0.1mM EDTA, 0.25mM DTT, 0.1mM PMSF, and 25% glycerol.

Storage and Stability

Store product at –70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

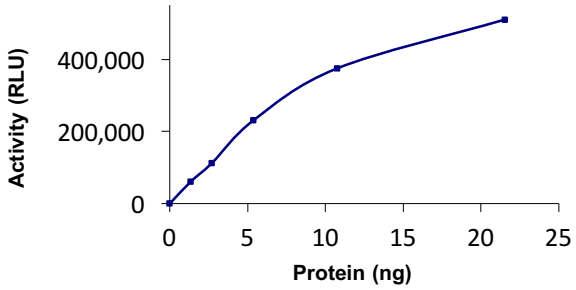
Scientific Background

SAE1 and UBA2 form a heterodimer that functions as a SUMO-activating enzyme for the sumoylation of proteins to regulate protein structures and intracellular localization (1). The SAE1/SAE2 dimer functions in SUMO1 activation in a manner analogous to the single E1 ubiquitin-activating enzymes (2). The SAE2 inactivation may be a therapeutic strategy in MYC-driven cancers (3).

References

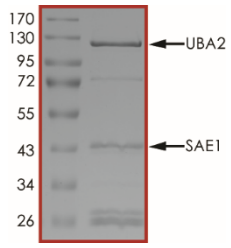
1. Okuma, T. et.al: In vitro SUMO-1 modification requires two enzymatic steps, E1 and E2. Biochem. Biophys. Res. Commun. 254: 693-698, 1999.
2. Desterro, J. et. al: Identification of the enzyme required for activation of the small ubiquitin-like protein SUMO-1. J. Biol. Chem. 274: 10618-10624, 1999.
3. Kessler, J. D. et.al: A SUMOylation-dependent transcriptional subprogram is required for Myc-driven tumorigenesis. Science 335: 348-353, 2012.

Specific Activity



The specific activity of SAE1/UBA2 was determined to be **45 nmol /min/mg** as per activity assay protocol.

Purity



The purity of SAE1/UBA2 complex was determined to be **>75%** by densitometry. The SAE1 was approx. MW **39kDa** and UBA2 was approx. MW **115kDa**.

SAE1/UBA2 Complex, Active

Recombinant full-length human protein expressed in Sf9 cells

Catalog #	U208-380G
Specific Activity	45 nmol/min/mg
Lot #	*D2411-3*
Purity	>75%
Concentration	0.1 µg/µl
Stability	1yr at –70°C from date of shipment
Storage & Shipping	Store product at –70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles. Product shipped on dry ice.

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Activity Assay Protocol

Reaction Components

Active Enzymes

Active SAE1/UBA2 (Catalog #: U208-380G) and UBE2I (Catalog #: U224-380H) diluted with SUMOylation Buffer and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of Active SAE1/UBA2 for optimal results).

SUMOylation Buffer

Buffer components: 50mM Tris-HCl (pH7.5), 5mM $MgCl_2$. Add 0.5mM DTT prior to use.

AMP-Glo™ Assay (Promega, Catalog #: V5011)

AMP, 10 mM
Ultra Pure ATP, 10mM
AMP-Glo™ Reagent I
AMP-Glo™ Reagent II
Kinase-Glo™ One Solution

Substrate

Human SUMO2 (1-93) (Catalog #: S294-31H) and RanGAP1 (Catalog #: R298-31H) diluted with SUMOylation Buffer to appropriate working stocks.

Assay Protocol

The SAE1/UBA2 assay is performed using the AMP-Glo™ Assay kit (Promega), by detecting the amount of the universal AMP generated. Ubiquitin-like (UBL) protein conjugation is proportional to the amount of generated AMP, and the presence of all components of the UBLs conjugation machinery (UBL, E1, E2 and substrate) is required for maximal activity of the system.

Step 1. Thaw the active SAE1/UBA2, UBE2I and SUMO2, RanGAP1 on ice, and all AMP-Glo™ components except AMP-Glo™ Reagent II at room temperature. Keep AMP-Glo™ Reagent II on ice.

Step 2. Prepare the following working solutions with SUMOylation Buffer:

- 2X Reaction Cocktail: 460ng/µl SUMO2 + 15ng/µl UBE2I + 80ng/µl RanGAP1 + 50µM ATP
- 2X final concentration of Active SAE1/UBA2

Step 3. In a half-area white 96-well plate, add the following components to bring the initial reaction volume to 10 µl:

Component 1. 5 µl of 2X Reaction Cocktail

Component 2. 5 µl of 2X Active SAE1/UBA2

Note: A blank control can be set up as outlined above by replacing the enzyme working solution with an equal volume of SUMOylation Buffer.

Step 4. Briefly centrifuge the plate to ensure reagents are fully mixed and at the bottom of the wells. Seal the plate with a plate seal and incubate at 30°C for 2 hours

Step 5. Equilibrate plate to room temperature. Add 10 µl of AMP-Glo™ Reagent I to all wells, mix by shaking for 1-2 minutes. Incubate the plate at room temperature for 60 minutes.

Step 6. Prepare AMP Detection Solution by adding AMP-Glo™ Reagent II to Kinase-Glo™ One Solution at a 1:100 volume ratio. Add 20 µl of the Detection Solution to all wells. Mix for 1-2 minutes and incubate at room temperature for 30 minutes

Step 7. Read the plate using the KinaseGlo Luminescence Protocol on a GloMax plate reader (Promega; Cat# E7031)

Step 8. Using the AMP standard curve, determine the concentration of AMP produced (µM) and calculate the enzyme specific activity as outlined below. For a detailed protocol of how to determine AMP amount from RLUs, see AMP-Glo™ Assay protocol at Promega's website: www.promega.com/protocols

Enzyme Specific Activity (SA) (nmol/min/mg)

$$= \frac{[AMP](\mu M) \times Reaction\ Volume(\mu l)}{Reaction\ Time\ (min) \times Enzyme\ Amount\ (mg)} \times 10^{-3}$$