Recombinant Human SUMO1-Specific Peptidase 1/SENP1

Catalog Number: 6587-SP

Background

The small ubiquitin-like modifier (SUMO) is a member of ubiquitin-like protein family. SUMO modification of the target proteins is a reversible process that regulates many cellular processes including transcription regulation, nuclear localization, centromere segregation and signal transduction (1). Sentrin-specific proteases (SENPs) are a group of cysteine-type peptidases that catalyze two essential functions in the SUMO pathways: processing of full-length SUMOs to their mature forms and deconjugation of SUMOs from SUMOlyated proteins (2). The seven mammalian SENPs share a conserved C-terminal catalytic domain while the N-terminal domains have no significant similarity. Human SENP-1 has broad specificity for the three mammalian SUMOs (3). It is found in the cytoplasm and nucleus depending on cell type, and is expressed in testis, thymus, pancreas, spleen, liver, ovary and small intestine. It is thought that localization of SENP-1 is vital for the regulation for SUMOylation status of target proteins. The recombinant human SENP-1 represents the catalytic domain, which has been shown to be sufficient for SENP-1 activity and substrate specificity (3, 4).

References:

1. Johnson, E.S. (2004) Annu. Rev. Biochem. **73**:355.
2. Drag, M. and G.S. Salvesen (2008) IUBMB Life. **60**:734.
3. Mikolajczyk, J. et al. (2007) J. Biol. Chem. **282**:26217.
4. Xu, Z. and S.W.N. Au (2005) Biochem. J. **386**:325.

Description

Source	*E. coli*-derived Glu419 - Leu644, with an N-terminal Met and 6-His tag. Accession # Q9P0U3
N-terminal Sequence Analysis	Met
Predicted Molecular Mass	28 kDa

Specifications

SDS-PAGE	27 - 28 kDa, reducing conditions
Activity	Measured by its ability to proteolytically process pro-SUMO1. <10 ng of rhSENP1 is required to cleave 50% of 1 µg of pro-SUMO1 or <25 ng of rhSENP1 is required to cleave 95% of 1 µg of pro-SUMO1, as measured under the described conditions. See Activity Assay Protocol.
Endotoxin Level	<1.0 EU per 1 µg of the protein by the LAL method.
Purity	>95%, by SDS-PAGE under reducing conditions and visualized by Colloidal Coomassie® Blue stain at 5 µg per lane.
Formulation	Supplied as a 0.2 µm filtered solution in Tris, NaCl, Glycerol, Brij-35 and DTT. See Certificate of Analysis for details.

Preparation and Storage

Shipping	The product is shipped with dry ice or equivalent. Upon receipt, store it immediately at the temperature recommended below.
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** ● 6 months from date of receipt, -70 °C as supplied. ● 3 months, -70 °C under sterile conditions after opening.

* Coomassie is a registered trademark of Imperial Chemical Industries Ltd.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Tris, 5 mM DTT, pH 8.5
- Recombinant Human SENP1 (rhSENP1) (R&D Systems, Catalog # 6587-SP)
- Recombinant Human SUMO1 (rhSUMO1) (R&D Systems, Catalog # 3289-SU)
- SDS-PAGE and/or Western blot
- Densitometer (BioRad GS-800 or equivalent)

Assay

1. Dilute rhSUMO1 to 100 µg/mL in Assay Buffer.
2. Prepare a curve of rhSENP1 in Assay Buffer. Make the following serial dilutions: 4, 2, 1, 0.5, 0.25, 0.125, and 0.0625 µg/mL.
3. Combine 10 µL of each dilution with 10 µL of the 100 µg/mL rhSUMO1. Include an enzyme control containing Assay Buffer in place of rhSENP1.
4. Incubate the reaction mixtures and control at 37 °C for 20 minutes.
5. Combine 20 µL of reactions (and controls) with 20 µL reducing SDS-PAGE sample buffer.
6. Analyze the cleavage by SDS-PAGE (load 40 µL per lane) followed by protein staining and/or Western blot.
7. Determine the % of full length cleaved by densitometry and plot vs. rhSENP1 concentration with 4-PL fitting.
8. Activity Calculation:

$$\% \text{ Cleavage} = \left[1 - \frac{\% \text{ full-length rhSUMO1 (reaction)}}{\% \text{ full-length rhSUMO1 (control)}}\right] \times 100\%$$

Final Assay Conditions Per Well

- rhSUMO1: 1 µg
- rhSENP1: 40, 20, 10, 5, 2.5, 1.25, 0.625, and 0 ng

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NOT FOR USE IN HUMANS