SUMO-2 (human, recombinant)

Cat. # SU202

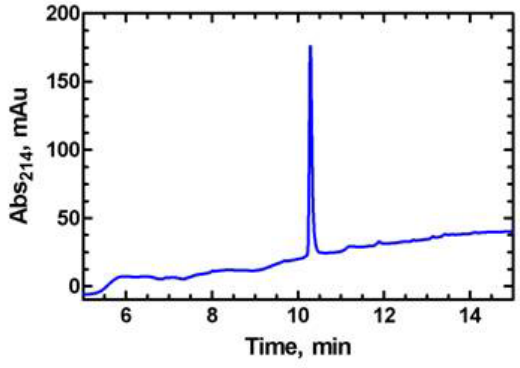
Background: Human recombinant SUMO-2 is produced in *E. coli* and purified to >95% homogeneity. This protein contains no extraneous tags. Vertebrates express three SUMO homologs, SUMO-1 and the closely related SUMO-2/3, which can be conjugated to substrate proteins in a manner analogous to the ubiquitin system[1-3]. Although a fourth SUMO (SUMO-4) has been identified in the genome[4], it may be an unexpressed pseudogene.

Application: For use in conjunction assays using SUMO E1 (Cat. # SU101) and UBE2I (Ubc9; Cat. # UB228H) (see protocol below). SUMO can be conjugated to consensus sequences (ψ-K-X-D/E; ψ is any large hydrophobic residue and X is any amino acid) *in vitro* independent of an E3 enzyme. Inclusion of a SUMO E3 can increase the rate of conjugation and/or direct SUMOylation to nonconsensus sites.

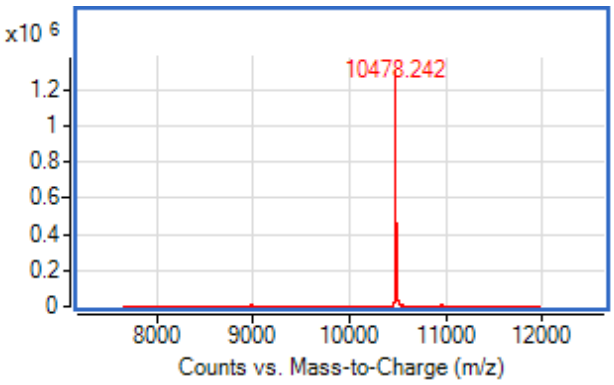
Alternative Names: Sentrin-2; Small ubiquitin-related modifier 2

Product Information

Accession No.:	P61956
Tag:	None
Expression Host:	*E. coli*
Purity:	≥ 95% by RP-HPLC
Molecular Weight:	10,477.7 Da (calculated)
Physical State:	5mg/ml in PBS
Quantity:	500 µg
Storage:	-80°C. Avoid repeated freeze/thaw cycles



RP-HPLC



Deconvoluted mass spectrum

References

1. Gareau, J.R. and C.D. Lima, *The SUMO pathway: emerging mechanisms that shape specificity, conjugation and recognition.* Nat Rev Mol Cell Biol, 2010. **11**(12): p. 861-71.
2. Cubenas-Potts, C. and M.J. Matunis, *SUMO: a multifaceted modifier of chromatin structure and function.* Dev Cell, 2013. **24**(1): p. 1-12.
3. Bettermann, K., et al., *SUMOylation in carcinogenesis.* Cancer Lett, 2012. **316**(2): p. 113-25.
4. Bohren, K.M., et al., *A M55V polymorphism in a novel SUMO gene (SUMO-4) differentially activates heat shock transcription factors and is associated with susceptibility to type I diabetes mellitus.* J Biol Chem, 2004. **279**(26): p. 27233-8.

Protocol:

E3 Ligase-independent SUMOylation Assay

1) Dilute the following into 10ul SUMOylation Assay Buffer:
    a. 0.2ug SUMO-1 or SUMO-2/3 (Cat. # SU201)
    b. 150ng SUMO E1 (Cat. # SU101)
    c. 200ng UBE2I (Ubc9; Cat. # UB228H)
    d. 0.2ug target substrate (150nM for a 65kDa protein)
    e. Add SUMOylation Assay Buffer to bring to 20ul final volume
2) Add 1uM of 100mM ATP
3) Incubate for 30-60minutes at 30^0C
4) Stop reaction with 20ul SDS sample buffer
5) Denature 3 minutes at 95^0C
6) Load 20ul onto an SDS-PAGE gel and analyze by Western Blotting. SUMOylation results in a shift in migration of your protein of 20kDa

SUMOylation Assay Buffer
20mM HEPES, pH7.3
110mM potassium acetate
2mM magnesium acetate
1mM EGTA
1mM DTT
0.05% Tween 20
0.2mg/ml ovalbumin
Optional: 1ug/ml leupeptin, pepstatin, aprotinin

ATP
100mM in 20mM HEPES, pH7.4, 100mM MgOAc