

SARS-CoV 3CL Protease, Recombinant from *E. coli*

Catalog No. NR-700

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Contributor:

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Product Description:

The SARS-CoV 3CL protease is a cysteine protease required for the proteolytic processing of the viral precursor polyprotein into the functional proteins required for replication. A homology model for the structure of the SARS-CoV 3CL protease has been constructed based on the crystal structures of other coronavirus proteases.¹

NR-700 was expressed and purified using a novel SUMO fusion system.^{2,4} An N-terminal histidine-tagged SUMO-3CL protease fusion was expressed in *E. coli* and purified by nickel affinity chromatography. After the fusion was cleaved by the SUMO protease, the SUMO tag and the SUMO protease (both histidine-tagged) were subtracted from the 3CL protease by nickel affinity chromatography. The 3CL protease was further purified by anion exchange chromatography, dialyzed against 10 mM ammonium bicarbonate, aliquoted and lyophilized. NR-700 has a molecular weight of approximately 33,800 daltons. The predicted sequence, protein properties and amino acid content of the 3CL protease are shown in Tables 1–3 below.

Material Provided:

Each vial contains approximately 1.0 mg of NR-700 lyophilized in 10 mM ammonium bicarbonate.

Packaging/Storage:

NR-700 was packaged aseptically in cryovials. The product is provided on dry ice and should be placed at -20°C or colder for long-term storage. Lyophilized NR-700 is stable for several weeks at 4°C.

Functional Activity:

Using Western blot analysis, NR-700 reacted with rabbit polyclonal sera prepared against the SUMO 3CL protease fusion, but did not react with rabbit polyclonal sera prepared against a SUMO nucleocapsid fusion.²

Citation:

Acknowledgment for publications should read "The following reagent was obtained through the NIH Biodefense and Emerging Infections Research Resources Repository, NIAID,

NIH: SARS-CoV 3CL Protease, Recombinant from *E. coli*, NR-700."

Biosafety Level: 1

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. Biosafety in Microbiological and Biomedical Laboratories. 4th ed. Washington, DC: U.S. Government Printing Office, 1999. HHS Publication No. (CDC) 93-8395. This text is available online at www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm.

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References:

1. Anand, K., et al. "Coronavirus Main Proteinase (3CL^{pro}) Structure: Basis for Design of Anti-SARS Drugs." Science 300 (2003): 1763–1767. PubMed: 12746549.
2. Zuo, X., et al. "Expression and Purification of SARS Coronavirus Proteins using SUMO-fusions." Protein

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3. Butt, T. R., S. C. Edavettal, J. P. Hall, and M. R. Mattern. "SUMO Fusion Technology for Difficult-to-express Proteins." Protein Express. Purif. 43 (2005): 1–9. PubMed: 16084395.
 4. Malakhov, M. P., et al. "SUMO Fusions and SUMO-specific Protease for Efficient Expression and Purification of Proteins." J. Struct. Funct. Genomics 5 (2004): 75–86. PubMed: 15263846.

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1	SGFRKMAFPS	GKVEGCMVQV	TCGTTTLNGL	WLDDEVYCP	HVICTAEDML
51	NPNYEDLLIR	KSNHSFLVQA	GNVQLRVIGH	SMQNCLLRK	VDTSNPKTPK
101	YKQVRIQPGQ	TFSVLACYNG	SPSGVYQCAM	RPNHTIKGSF	LNGSCGSVGF
151	NIDYDCVSFC	YMHHMELPTG	VHAGTDLEGK	FYGPVDRQT	AQAAGTDTTI
201	TLNVLAWLYA	AVINGDRWFL	NRFTTTLNDF	NLVAMKYNYE	PLTQDHVDIL
251	GPLSAQTGIA	VLDMCAALKE	LLQNGMNGRT	ILGSTILEDE	FTPFDVVRQC
301	SGVTFQ				

Length	306 amino acids
Molecular weight	33843 daltons
1 microgram	29.5 pmoles
Molar extinction coefficient	32590
1 A[280]	1.04 mg/mL
A[280] of 1 mg/mL	0.96 AU
Isoelectric point	6.24
Charge at pH 7	-2.64

Amino Acids	Count	% by Weight	% by Frequency
Charged (RKHYCDE)	80	30.43	26.14
Acidic (DE)	26	9.12	8.50
Basic (KR)	23	9.40	7.52
Polar (NCQSTY)	98	32.49	32.03
Hydrophobic (AILFWV)	102	33.28	33.33
A Ala	17	3.85	5.56
C Cys	12	3.70	3.92
D Asp	17	5.75	5.56
E Glu	9	3.37	2.94
F Phe	16	6.72	5.23
G Gly	26	4.96	8.50
H His	8	3.16	2.61
I Ile	12	4.00	3.92
K Lys	11	4.09	3.59
L Leu	30	10.00	9.80
M Met	10	3.79	3.27
N Asn	19	6.38	6.21
P Pro	13	3.80	4.25
Q Gln	14	5.20	4.58
R Arg	12	5.31	3.92
S Ser	16	4.27	5.23
T Thr	26	7.87	8.50
V Val	24	7.15	7.84
W Trp	3	1.56	0.98
Y Tyr	11	5.06	3.59
B Asx	36	12.13	11.76
Z Glx	23	8.57	7.52