SARS-CoV Nucleocapsid (N) Protein, Recombinant from *E. coli*

Catalog No. NR-699

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Contributor:

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Product Description:

The SARS-CoV nucleocapsid (N) protein is a phosphoprotein that binds the viral RNA inside the virion. The N protein bears a high similarity to the nucleocapsid proteins of other coronaviruses. However, it contains a short unique lysine-rich region (KTFPPTEPKKDKKKKTDEAQ) that is not found in any other viruses. The function of this region is not known; however, it is speculated that it may be involved in pathogenesis.[1] The highly basic nature of this peptide is characteristic of an RNA-binding protein. The N protein is known to have B and T cell epitopes and to elicit host protective immune responses.

NR-699 was expressed and purified using a novel SUMO fusion system.[2–4] An N-terminal histidine-tagged SUMO-nucleocapsid 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 nucleocapsid by nickel affinity chromatography. The nucleocapsid was further purified by cation exchange chromatography, dialyzed against 10 mM ammonium bicarbonate, aliquoted and lyophilized. NR-699 has a molecular weight of approximately 46,000 daltons. The predicted sequence, protein properties and amino acid content of SARS-CoV nucleocapsid are shown in Tables 1–3 below.

Material Provided:

Each vial contains approximately 1.0 mg of NR-699 lyophilized in 10 mM ammonium bicarbonate.

Packaging/Storage:

NR-699 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-699 is stable for several weeks at 4°C.

Functional Activity:

Using Western blot analysis, NR-699 reacted with rabbit polyclonal sera prepared against the SUMO nucleocapsid fusion, but did not react with rabbit polyclonal sera prepared against a SUMO-3CL protease 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 Nucleocapsid (N) Protein, Recombinant from *E. coli*, NR-699."

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. Marra, M. A., et al. "The Genome Sequence of the SARS-Associated Coronavirus." Science 300 (2003): 1399–1404. PubMed: 12730501.
2. Zuo, X., et al. "Expression and Purification of SARS Coronavirus Proteins using SUMO-fusions." Protein Expr. Purif. 42 (2005): 100–110. PubMed: 15939295.
3. Butt, T. R., S. C. Edavettal, J. P. Hall, and M. R. Mattern. "SUMO Fusion Technology for Difficult-to-express Proteins." Protein Expr. 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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Table 1 – Predicted Protein Sequence						
1	MSDNGPQSNQ	RSAPRITFGG	PTDSTDNNQN	GGRNGARPKQ	RRPQGLPNNT
51	ASWFTALTQH	GKEELRFPRG	QGVPINTNSG	PDDQIGYYRR	ATRRVRGGDG
101	KMKELSPRWY	FYYLGTGPEA	SLPYGANKEG	IVWVATEGAL	NTPKDHIGTR
151	NPNNNAATVL	QLPQGTTLPK	GFYAEGSRGG	SQASSRSSSR	SRGNSRNSTP
201	GSSRGNSPAR	MASGGGETAL	ALLLLDRLNQ	LESKVSGKGQ	QQQGQTVTKK
251	SAAEASKKPR	QKRTATKQYN	VTQAFGRRGP	EQTQGNFGDQ	DLIRQGTDYK
301	HWPQIAQFAP	SASAFFGMSR	IGMEVTPSGT	WLTYHGAIKL	DDKDPQFKDN
351	VILLNKHIDA	YKTFPPTEPK	KDKKKKTDEA	QPLPQRQKKQ	PTVTLLPAAD
401	MDDFSRQLQN	SMSGASADST	QA		

Table 2 – Predicted Protein Properties	
Length	422 amino acids
Molecular weight	46022 daltons
1 microgram	21.7 pmoles
Molar extinction coefficient	42530
1 A[280]	1.08 mg/mL
A[280] of 1 mg/mL	0.92 AU
Isoelectric point	10.11
Charge at pH 7	24.22

Table 3 – Predicted Amino Acid Content			
Amino Acids	**Count**	**% by Weight**	**% by Frequency**
Charged (RKHYCDE)	112	32.45	26.54
Acidic (DE)	36	9.30	8.53
Basic (KR)	60	17.98	14.22
Polar (NCQSTY)	138	33.34	32.70
Hydrophobic (AILFWV)	100	23.02	23.70
A Ala	34	5.65	8.06
C Cys	0	0.00	0.00
D Asp	22	5.46	5.21
E Glu	14	3.84	3.32
F Phe	13	4.01	3.08
G Gly	45	6.30	10.66
H His	5	1.45	1.18
I Ile	11	2.69	2.61
K Lys	29	7.91	6.87
L Leu	26	6.36	6.16
M Met	7	1.95	1.66
N Asn	25	6.16	5.92
P Pro	31	6.66	7.35
Q Gln	34	9.27	8.06
R Arg	31	10.07	7.35
S Ser	35	6.86	8.29
T Thr	33	7.33	7.82
V Val	11	2.40	2.61
W Trp	5	1.90	1.18
Y Tyr	11	3.72	2.61
B Asx	47	11.62	11.14
Z Glx	48	13.11	11.37

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