MRC PPU Reagents and Services

Standard Operating Procedure

Preparation of Nucleocapsid Protein [1 - 419] SARS CoV2

Enzyme description:- N Protein [1 - 419]

Clone number:- DU 67728

Source:- Recombinant

Expression system:- *E. coli*

Tag:- N-terminal MBP

Purification method:- Amylose Resin

Calculated molecular mass:-
Monoisotopic 89, 809.39 daltons
Average Mass 89, 864.61 daltons
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 8.97

Purity:- 80 %

Enzyme storage buffer:-
50 mM Tris-HCl pH 7.5, 270 mM Sucrose, 150 mM NaCl, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 0.03 % Brij-35

Storage temperature:- –70 °C

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Clone Data Sheet

Nucleocapsid Protein [1 – 419] SARS CoV2

Protein N Protein [1 - 419]

Clone number DU 67728

Accession number QHD43423.2

Tags N-terminal MBP

Bacterially expressed protein

MKIEEGKLVIWINGDKGYNGLAEVGKKFEKDTGIKVTVEHPDKLE
EKFPQVAATGDGPDIIFWAHDRFGGYAQSGLLAEITPDKAFQDKL
YPFTWDAVRYNGKLIAYPIAVEALSLIYNKDLLPNPPKTWEEIPA
LDKELKAKGKSALMFNLQEPYFTWPLIAADGGYAFKYENGKYDIK
DVGVDNAGAKAGLTFLVDLIKNKHMNADTDYSIAEAAFNKGETAM
TINGPWAWSNIDTSKVNYGVTVLPTFKGQPSKPFVGVLSAGINAA
SPNKELAKEFLENYLLTDEGLEAVNKDKPLGAVALKSYEEELVKD
PRIAATMENAQKGEIMPNIPQMSAFWYAVRTAVINAASGRQTVDE
ALKDAQTNSSSNNNNNNNNNLGDDDDKVPEFLEVLFQGPLGS**MS
DNGPQNQRNAPRITFGGPSDSTGSNQNGERSGARSKQRRPQGLPN
NTASWFTALTQHGKEDLKFPRGQGVPINTNSSPDDQIGYYRRATR
RIRGGDGKMKDLSPRWYFYYLGTGPEAGLPYGANKDGIIWVATEG
ALNTPKDHIGTRNPANNAAIVLQLPQGTTLPKGFYAEGSRGGSQA
SSRSSSRSRNSSRNSTPGSSRGTSPARMAGNGGDAALALLLLDRL
NQLESKMSGKGQQQQGQTVTKKSAAEASKKPRQKRTATKAYNVTQ
AFGRRGPEQTQGNFGDQELIRQGTDYKHWPQIAQFAPSASAFFGM
SRIGMEVTPSGTWLTYTGAIKLDDKDPNFKDQVILLNKHIDAYKT
FPPTEPKKDKKKKADETQALPQRQKKQQTVTLLPAADLDDFSKQL
QQSMSSADSTQA**

Native sequence Amino acids M1 – A419 (end).
Residue M404 of the fusion protein is equivalent to M1 of the native enzyme. The MBP tag is located at residues 1 – 367.

Protease cleavage Enterokinase (DDDDK) residues 384 – 388
PreScission (LEVLFQGP) residues 393 – 400