MRC PPU Reagents and Services

Standard Operating Procedure

Preparation of NSP4 [386 - 500] SARS CoV2

Enzyme description:- NSP4 [386 - 500]

Clone number:- DU 61988

Source:- Recombinant

Expression system:- *E. coli*

Tag:- N-terminal MBP

Purification method:- Amylose Resin

Calculated molecular mass:-
Monoisotopic 57, 537.00 daltons
Average Mass 57, 573.14 daltons
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.23

Purity:- 85 %

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

NSP4 [386 – 500] SARS CoV2

Protein NSP4 [386 - 500]

Clone number DU 61988

Accession number QHD43415.1

Tags N-terminal MBP

Bacterially expressed protein

MKIEEGKLVIWINGDKGYNGLAEVGKKFEKDTGIKVTVEHPDKLE
EKFPQVAATGDGPDIIFWAHDRFGGYAQSGLLAEITPDKAFQDKL
YPFTWDAVRYNGKLIAYPIAVEALSLIYNKDLLPNPPKTWEEIPA
LDKELKAKGKSALMFNLQEPYFTWPLIAADGGYAFKYENGKYDIK
DVGVDNAGAKAGLTFLVDLIKNKHMNADTDYSIAEAAFNKGETAM
TINGPWAWSNIDTSKVNYGVTVLPTFKGQPSKPFVGVLSAGINAA
SPNKELAKEFLENYLLTDEGLEAVNKDKPLGAVALKSYEEELVKD
PRIAATMENAQKGEIMPNIPQMSAFWYAVRTAVINAASGRQTVDE
ALKDAQTNSSSNNNNNNNNNLGDDDDKVPEFLEVLFQGPLGS**ST
KHFYWFFSNYLKRRVVFNGVSFSTFEEAALCTFLLNKEMYLKLRS
DVLLPLTQYNRYLALYNKYKYFSGAMDTTSYREAACCHLAKALND
FSNSG SDVLYQPPQTSITSAVLQ**

Native sequence Amino acids S386 – Q500 (end residue).
Residue S404 of the fusion protein is equivalent to S386 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