

MRC PPU Reagents and Services

Standard Operating Procedure

Preparation of NSP8 [1 - 198] SARS CoV2

Enzyme description:- NSP8 [1 - 198]

Clone number:- DU 66418

Source:- Recombinant

Expression system:- *E. coli*

Tag:- N-terminal MBP

Purification method:- Amylose Resin

Calculated molecular mass:-

Monoisotopic 66,078.48 daltons

Average Mass 66,120.00 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.01

Purity:- 95 %

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

MRC PPU Reagents and Services

Clone Data Sheet

NSP8 [1 – 198] SARS CoV2

Protein NSP8 [1 - 198]

Clone number DU 66418

Accession number QHD43415.1

Tags N-terminal MBP

**Bacterially
expressed protein**

MKIEEGKLVIIWINGDKGYNGLAEVGGKFEKDTGIKVTVEHPDKLE
EKFPQVAATGDGPDIIFWAHDRFGGYAQSGLLAEITPDKAFQDKL
YFPTWDAVRYNGKLIAYPIAVEALSIIYNKDLLPNPPKTWEEIIPA
LDKELKAKGKSALMFNLQEPYFTWPLIAADGGYAFKYENKDYDIK
DVGVDNAGAKAGLTFLVDLIKNKHMNADTDYSIAEAAFNKGETAM
TINGPWAWSNIDTSKVNYGVTVLPTFKGQPSKPFVGVLSAGINAA
SPNKELAKEFLENYLLTDEGLEAVNKDKPLGAVALKSYYEELVKD
PRIAATMENAQKGEIMPNI PQMSAFWYAVRTAVINAASGRQTVDE
ALKDAQTNSSSNNNNNNNNNLGD~~DDDK~~VPEFLEVL~~FQGP~~LSAI
ASEFSSLPSYAAFATAQEA~~YEQ~~AVANGDSEVVLK~~LK~~SLNVAKS
EFDRDAAMQRKLEK~~MADQ~~AMT~~Q~~MY~~Q~~ARSEDKRAKVTSAM~~Q~~TMLF
TMLRKLNDALNNIINNARDGCVPLNIIP~~LT~~TAAKLMVVI~~P~~DYNT
YKNTCDGTTFTYASALWEIQ~~Q~~VVDADSKIV~~Q~~LSEISMDNSPNLAW
PLIVTALRANS~~AV~~KLQ

Native sequence Amino acids A1 – Q198 (end).
Residue A404 of the fusion protein is equivalent to A1 of the native enzyme. The MBP tag is located at residues 1 – 367.

Protease cleavage Enterokinase (DDDDK) residues 384 – 388
PreScission (LEVL~~FQGP~~) residues 393 – 400