

MRC PPU Reagents and Services

Standard Operating Procedure

Preparation of ORF8 [1 - 121] SARS CoV2

Enzyme description:- ORF8 [1 - 121]

Clone number:- DU 68496

Source:- Recombinant

Expression system:- *E. coli*

Tag:- N-terminal MBP

Purification method:- Amylose Resin

Calculated molecular mass:-

Monoisotopic 58,033.24 daltons

Average Mass 58,069.92 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 4.94

Purity:- 75 %

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

ORF8 [1 – 121] SARS CoV2

Protein ORF8 [1 - 121]

Clone number DU 68496

Accession number QHD43422.1

Tags N-terminal MBP

**Bacterially
expressed protein**

MKIEEGKLVIIWINGDKGYNGLAEVGGKFEKDTGIKVTVEHPDKLE
EKFPQVAATGDGPDIIFWAHDRFGGYAQSGLLAEITPDKAFQDKL
YFFTWDVAVRYNGKLIAYPIAVEALSLIYNKDLLPNPPKTWEEIPA
LDKELKAKGKSALMFNLQEPYFTWPLIAADGGYAFKYENGYDIK
DVGVDNAGAKAGLTFLVDLIKNKHMNADTDYSIAEAAFNKGETAM
TINGPWAWSNIDTSKVNYGVTVLPTFKGQPSKPFVGVLSAGINAA
SPNKELAKEFLENYLLTDEGLEAVNKDKPLGAVALKSYYEELVKD
PRIAATMENAQKGEIMPNIPOMSAFWYAVRTAVINAASGRQTVDE
ALKDAQTNSSNNNNNNNNNNLGDGDDDKVPEFLEVLFGQPLGSMK
FLVFLGIITTVAAFHQECSLQSQCTQHQPYPVDDPCPIHFYSKWYI
RVGARKSAPLIELCVDEAGSKSPIQYIDIGNYTVSCLPFTINCQE
PKLGSLVVRCSFYEDFLEYHDVRRVLDFI

Native sequence Amino acids M1 – I121 (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