

MRC PPU Reagents and Services

Standard Operating Procedure

Preparation of ORF3B [1 - 151] SARS CoV2

Enzyme description:- ORF3B [1 - 151]

Clone number:- DU 68511

Source:- Recombinant

Expression system:- *E. coli*

Tag:- N-terminal MBP

Purification method:- Amylose Resin

Calculated molecular mass:-

Monoisotopic 61, 507.47 daltons

Average Mass 61, 546.19 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 4.77

Purity:- 70 %

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

ORF3B [1 – 151] SARS CoV2

Protein ORF3B [1 - 151]

Clone number DU 68511

Accession number QHD43419.1

Tags N-terminal GST

Bacterially expressed protein

MKIEEGKLVWINGDKGYNGLAIEVGGKFEKDTGIKVTVEHPDKLE
EKFPQVAATGDGPDIIFWAHDRFGGYAQSGLLAEITPDKAFQDKL
YPFTWDAVRYNGKLIAYPIAVEALSLIYNKDLLPNPPKTWEEIPA
LDKELKAKGKSALMFNLQEPYFTWPLIAADGGYAFKYENKGYDIK
DVGVDNAGAKAGLTFLVDLIKNKHMNADTDYSIAEAAFNKGETAM
TINGPWAWSNIDTSKVNYGVTVLPTFKGQPSKPFVGVLSAGINAA
SPNKELAKEFLENYLLTDEGLEAVNKDKPLGAVALKSYYYEELVKD
PRIAATMENAQKGEIMPNIPOMSAFWYAVRTAVINAASGRQTVDE
ALKDAQTNSSNNNNNNNNNNLGDGDDDKVPEFLEVLFGQPLGSMR
LWLCWKCRSKNPLLYDANYFLCWHTNCYDYCIPYNSVTSSIVITS
GDGTTSPISEHDYQIGGYTEKWESGVKDCVVLHSYFTSDYYQLYS
TQLSTDTGVEHVTFFIYNKIVDEPEEHVQIHTIDGSSGVVNPVME
PIYDEPTTTTSVPL

Native sequence Amino acids M1 - L151 (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 (LEVLFGQP) residues 393 – 400