

MRC PPU Reagents and Services

Standard Operating Procedure

Preparation of ORF3A [1 - 275] SARS CoV2

Enzyme description:- ORF3A [1 - 275]

Clone number:- DU 67722

Source:- Recombinant

Expression system:- *E. coli*

Tag:- N-terminal MBP

Purification method:- Amylose Resin

Calculated molecular mass:-

Monoisotopic 75,314.19 daltons

Average Mass 75,361.85 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.03

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

ORF3A [1 – 275] SARS CoV2

Protein ORF3A [1 - 275]

Clone number DU 67722

Accession number QHD43417.1

Tags N-terminal MBP

**Bacterially
expressed protein**

MKIEEGKLVIWINGDKGYNGLAEVGGKFEKDTGIKVTVEHPDKLE
EKFPQVAATGDGPDIIFWAHDRFGGYAQSGLLAEITPDKAFQDKL
YPFTWDAVRYNGKLIAYPIAVEALSLIYNKDLLPNPPKTWEEIPA
LDKELKAKGKSALMFNLQEPYFTWPLIAADGGYAFKYENGYDIK
DVGVDNAGAKAGLTFVLVDLIKKNHMNADTDYSIAEAAFNKGETAM
TINGPWAWSNIDTSKVNYGVTVLPTFKGQPSKPFVGVLSAGINAA
SPNKELAKEFLENYLLTDEGLEAVNKDKPLGAVALKSYYYEELVKD
PRIAATMENAQKGEIMPNIPOMSAFWYAVRTAVINAASGRQTVDE
ALKDAQTNSSNNNNNNNNNNLGDGDDDKVPEFLEVLFGQPLGSM
LFMRIFTIGTVTLKQGEIKDATPSDFVRATATIPIQASLPFGWLI
VGVALLAVFQSASKIITLKKRWQLALSKGVHFVCNLLLLFVTVYS
HLLLVAAGLEAPFLYLYALVYFLQSINFRVIMRLWLCWKCRSKN
PLLYDANYFLCWHTNCYDYCIPYNSVTSSIVITSGDGTSPISEH
DYQIGGYTEKWESGVKDCVVLHSYFTSDYYQLYSTQLSTDTGVEH
VTFFIYNKIVDEPEEHVQIHTIDGSSGVNPNVMEPIYDEPTTTTS
VPL

Native sequence Amino acids M1 – L275 (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