

MRC PPU Reagents and Services

Standard Operating Procedure

Preparation of NSP5 [1 - 306] SARS CoV2

Enzyme description:- NSP5 [1- 306]

Clone number:- DU 67779

Source:- Recombinant

Expression system:- *E. coli*

Tag:- N-terminal GST

Purification method:- GSH Agarose

Calculated molecular mass:-

Monoisotopic 60, 581.13 daltons

Average Mass 60, 620.81 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.84

Purity:- 90 %

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, 0.2 mM PMSF, 1 mM Benzamidine

Storage temperature:- -70 °C

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Clone Data Sheet

NSP5 [1 – 306] SARS CoV2

Protein NSP5 [1 - 306]

Clone number DU 67779

Accession number QHD43415.1

Tags N-terminal GST

**Bacterially
expressed protein**

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKK
FELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERA
EISMLEGAVLDIRYGVSR IAYS KDFETLKVDFLSKLPEMLKMFED
RLCHKTYLNGDHVTHPDMFLYDALDVVLYMDPMCLDAFPKLVCFK
KRIEAIPOIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSDLEVL
FQGPLGSSGFRKMAFPSGKVEGCMVQVTCGTTTLNGLWLDVVYCP
RHVICTSEDMLNPNYEDLLIRKSNHNFLVQAGNVQLRVIGHSMQN
CVLKLKVD TANPKTPKYKFVRIQPGQTF SVLACYNGSPSGVYQCA
MRPNFTTIKGSFLNGSCGSVGFNIDYDCVSFCYMHMELPTGVHAG
TDLEGNFYGPFVDRQTAQAAGTDTTITVNVLAWLYAAVINGDRWF
LNRFTTTLNDFNLVAMKYNYEPLTQDHVDILGPLSAQTGIAVLDM
CASLKELLQNGMNGRTILGSALLEDEFTPF DVVRQCSGVTFQ

Native sequence Amino acids S1 – Q306 (end).
Residue S232 of the fusion protein is equivalent to S1 of the native enzyme. The GST tag is located at residues 1 – 220.

Protease cleavage PreScission (LEVLFGQP) residues 221 - 228