

MRC PPU Reagents and Services

Standard Operating Procedure

Preparation of NSP5 [1 - 306] SARS CoV2

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|---|--|
| <u>Enzyme description:-</u> | NSP5 [1- 306] |
| <u>Clone number:-</u> | DU 67832 |
| <u>Source:-</u> | Recombinant |
| <u>Expression system:-</u> | <i>E. coli</i> |
| <u>Tag:-</u> | Both N-terminal GST and C-terminal His6 cleaved |
| <u>Purification method:-</u> | Cobalt Agarose |
| <u>Calculated molecular mass:</u> | |
| Monoisotopic | 33, 796.64 daltons [after cleavage of GST and His6 tag] |
| Average Mass | 33, 774.50 daltons [after cleavage of GST and His6 tag] [cysteines reduced, methionines have not been oxidised] |
| <u>Theoretical pI:-</u> | 5.95 |
| <u>Purity:-</u> | 90 % |
| <u>Enzyme storage buffer:-</u> | |
| 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 | |
| <u>Storage temperature:-</u> | -70 °C |

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Clone Data Sheet

NSP5 [1 – 306] SARS CoV2

Protein NSP5 [1 - 306]

Clone number DU 67832

Accession number QHD43415.1

Tags N-terminal GST and C-terminal His6 both cleaved

Bacterially expressed protein

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKK
FELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERA
EISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLPPEMLKMFED
RLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFK
KRIEAIPOIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSDLEVL
FQGPLGSSAVLQ**SGFRKMAFPSGKVEGCMVQVTCGTTTTLNGLWLD**
VVYCPRHVICTSEDMLNPNYEDLLIRKSNHNFLVQAGNVQLRVIG
HSMQNCVLKLVDTANPKTPKYKFVRIQPGQTFSVLACYNGSPSG
VYQCAMRPNFTIKGSFLNGSCGSVGFNIDYDCVSFCYMHMELPT
GVHAGTDLEGNFYGPFVDRQTAQAAGTDTTITVNVLAWLYAAVIN
GDRWFLNRFTTTLNDFNLVAMKYNIEPLTQDHVDILGPLSAQTGI
AVLDMCASLKELLQGMNGRTILGSALLEDEFTPFDVVRQCSGVT
FQGPHHHHHH

Native sequence Amino acids S1 – Q306 (end).
Residue S237 of the fusion protein is equivalent to S1 of the native enzyme. The GST tag is located at residues 1 – 220 and the His6 tag is located at residues 545– 550.

Has C-terminal 5' residues of NSP4 (residues 232 – 236) between PreScission site and N-terminus of NSP5 – corresponding to the cleavage site between NSP4 and NSP5 in the polyprotein of the SARS CoV virus to generate an authentic N-terminus during gene expression. The C-terminus encodes for a modified PreScission cleavage site before the His6 tag to generate an authentic C-terminus when cleaved, SGVTFQGP, residues 537 - 544.

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