

MRC PPU Reagents and Services

Standard Operating Procedure

Preparation of NSP11 NSP12 [1 - 932] SARS CoV2

Enzyme description:- NSP11 NSP12 [1 - 932] SARS CoV2

Clone number:- DU 67736

Source:- Recombinant

Expression system:- *E. coli*

Tag:- N-terminal GST

Purification method:- GSH Agarose

Calculated molecular mass:-

Monoisotopic 133,397.72 daltons

Average Mass 133,484.40 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 6.05

Purity:- 60 %

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

MRC PPU Reagents and Services

Clone Data Sheet

NSP11 NSP12 [1 – 932] SARS CoV2

Protein NSP11 NSP12 [1 - 932]

Clone number DU 67736

Accession number QHD43415.1

Tags N-terminal GST

Bacterially
expressed protein

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKK
FELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERA
EISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLPPEMLKMFED
RLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFK
KRIEAIPOIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSDLEVL
FQGPLGSSADAQSFLNRVCGVSAARLTPCGTGTSTDVVYRAFDIYN
DKVAGFAKFLKTNCCRFQEKDEDDNIDSYFVVKRHTFSNYQHEE
TIYNLLKDCPAVAKHDFKFRIDGDMVPHISRQRLTKYTMADLVY
ALRHFDEGNCDTLKEILVTYNCCDDYFNKKDWYDFVENPDILRV
YANLGERVRQALLKTVQFCDAMRNAGIVGVLTLDNQDLNGNWYDF
GDFIQTTPGSGVPVVDSSYSLLMPIILTLTRALTAESHVDTDLTKP
YIKWDLKDYDFTEERLKLFDYFQYWDQTYHPNCVNCLEDDRCILH
CANFNVLFSSTVFPPTSFGPLVRKIFVDGVPFVSTGYHFRELGVV
HNQDVNLHSSRSLFKELLVYAADPAMHAASGNLLLDKRTTCFSVA
ALTNNVAFQTVKPGNFNKFYDFAVSKGFFKEGSSVELKHFFFAQ
DGNAAISDYDYRYNLPTMCDIRQLLFVVEVVDKYFDCYDGGCIN
ANQVIVNNLDKSAGFPFNKWKARLYYDSMSYEDQDALFAYTKRN
VIPTITQMNLKYAISAKNRARTVAGVSI CSTMNROFHQKLLKSI
AATRGATVVIIGTSKFYGGWHNMLKTVYSDVENPHLMGWDYPKCDR
AMPNMLRIMASLVLARKHTTCCSLSHRFYRLANCAQVLSEMVMC
GGSLYVKPGGTSSGDATTAYANSVFNICQAVTANVNALLSTDGKN
IADKYVRNLQHRLYECLYRNRDVTDFVNEFYAYLRKHF SMMILS
DDAVVCFNSTYASQGLVASIKNFKSVLYYQNNVFMSEAKWTETD
LTKGPHEFCSQHTMLVKQGGDYVYLPYPDPSRILGAGCFVDDIVK
TDGTLMIERFVSLAIDAYPLTKHPNQEYADVHFHLYLOYIRKLHDE
LTGHMLDMYSVMLTNDNTSRYWEPEFYEAMYTPHTVLQ

Native sequence

Amino acids S1 – Q932 (end).

Residue S232 of the fusion protein is equivalent to S1 of the native enzyme. The GST tag is located at residues 1 – 220.

MRC PPU Reagents and Services

Protease cleavage PreScission (LEVLFQGP) residues 221 – 228