

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 67744

Source:- Recombinant

Expression system:- *E. coli*

Tag:- N-terminal MBP

Purification method:- Amylose Resin

Calculated molecular mass:-

Monoisotopic 150, 802.46 daltons

Average Mass 150, 899.15 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.63

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

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Clone Data Sheet

NSP11 NSP12 [1 – 932] SARS CoV2

| | |
|--------------------------------------|--|
| <u>Protein</u> | NSP11 NSP12 [1 - 932] |
| <u>Clone number</u> | DU 67744 |
| <u>Accession number</u> | QHD43415.1 |
| <u>Tags</u> | N-terminal MBP |
| <u>Bacterially expressed protein</u> | <p>MKIEEGKLVIIWINGDKGYNGLAEVGGKFEKDTGIKVTVEHPDKLE EKFPQVAATGDGPDIIFWAHDRFGGYAQSGLLAEITPDKAFQDKL YPFTWDAVRYNGKLIAYPIAVEALSLIYNKDLLPNPPKTWEEIPA LDKELKAKGKSALMFNLQEPYFTWPLIAADGGYAFKYENGKYDIK DVGVDNAGAKAGLTFVLVDLIKKNHMNADTDYSIAEAAFNKGETAM TINGPWAWSNIDTSKVNYGVTVLPTFKGQPSKPFVGVLSAGINAA SPNKELAKEFLENYLLTDEGLEAVNKDKPLGAVALKSYYYEELVKD PRIAATMENAQKGEIMPNI PQMSAFWYAVRTAVINAASGRQTVDE ALKDAQTNSSSNNNNNNNNNLGDGDDKVPEFLEVLFOGPLGSSA DAQSFLNRVCGVSAARLTPCGTGTSTDVVYRAFDIYNDKVAGFAK FLKTNCCRFQEKDEDDNLDISYFVVKRHTFSNYQHEETIYNLLKD CPAVAKHDFFKFRIDGMVPHISRQRLTKYTMADLVYALRHFDEG NCDTLKEILVTYNCCDDDFNKKDWYDFVENPDILRVYANLGERV RQALLKTVQFCDAMRNAGIVGVLTLDNQDLNGNWDYDFGDFIQTP GSGVPVVDSSYLLMPILTLTRALTAESHVDTDLTKPYIKWDLK YDFTEERLKLFDYFYKYWDQTYHPNCVNCLEDDRCILHCANFNVL STVFPPTSFGPLVRKIFVDGVPFVSTGYHFRGLGVVHNQDVNLH SSRLSFKELLVYAADPAMHAASGNLLLDKRTTCFSVAALTNNVAF QTVKPGNFNKDFYDFAVSKGFFKEGSSVELKHFFFAQDGNAAISD YDYRYNLPMTCDIRQLLFVVEVVDKYFDCYDGGCINANQVIVNN LDKSAGFPFNKWKARLYYDSMSYEDQDALFAYTKRNVIPTITQM NLKYAISAKNRARTVAGVSI CSTMTNRQFHQKLLKSI AATRGATV VIGTSKFGYGGWHNMLKTVYSDVENPHLMGWDYPKCDRAMPNMLRI MASLVLARKHTTCCSLSHRFYRLANCAQVLSEMVMCGGSLYVKP GGTSSGDATTAYANSVFNICQAVTANVNALLSTDGNKIADKYVRN LQHRLYECLYRNRDVDTDVNEFYAYLRKHFSSMILSDDAVVCFN STYASQGLVASIKNFKSVLYYQNNVFMSEAKCWTETDLTKGPHEF CSQHTMLVKQGDDYVYLPYDPSPRILGAGCFVDDIVKTDGTLMI RFVSLAIDAYPLTKHPNQEYADVFLYLYQYIRKHLDELTGHMLDM YSVMLTNDNTSRYWEPEFYEAMYTPHTVLQ</p> |
| <u>Native sequence</u> | <p>Amino acids S1 – Q932 (end). Residue S404 of the fusion protein is equivalent to S1 of the native enzyme. The MBP tag is located at residues 1 – 367.</p> |

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Protease cleavage

Enterokinase (DDDDK) residues 384 – 388

PreScission (LEVLFQGP) residues 393 – 400