MRC PPU Reagents and Services

Standard Operating Procedure

Preparation of NSP13 [1 - 601] SARS CoV2

Enzyme description:- NSP13 [1 - 601]

Clone number:- DU 66417

Source:- Recombinant

Expression system:- *E. coli*

Tag:- N-terminal GST

Purification method:- GSH Agarose

Calculated molecular mass:-
Monoisotopic 93, 618.22 daltons
Average Mass 93, 678.91 daltons
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 8.24

Purity:- 80 %

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

NSP13 [1 – 601] SARS CoV2

Protein NSP13 [1 - 601]

Clone number DU 66417

Accession number QHD43415.1

Tags N-terminal GST

Bacterially expressed protein

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKK
FELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERA
EISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFED
RLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFK
KRIEAIPQIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLF
QGPLGS**AVGACVLCNSQTSLRCGACIRRPFLCCKCCYDHVISTSH
KLVLSVNPYVCNAPGCDVTDVTQLYLGGMSYYCKSHKPPISFPLC
ANGQVFGLYKNTCVGSDNVTDFNAIATCDWTNAGDYILANTCTER
LKLFAAETLKATEETFKLSYGIATVREVLSDRELHLSWEVGKPRP
PLNRNYVFTGYRVTKNSKVQIGEYTFEKGDYGDAVVYRGTTTYKL
NVGDYFVLTSHTVMPLSAPTLVPQEHYVRITGLYPTLNISDEFSS
NVANYQKVGMQKYSTLQGPPGTGKSHFAIGLALYYPSARIVYTAC
SHAAVDALCEKALKYLPIDKCSRIIPARARVECFDKFKVNSTLEQ
YVFCTVNALPETTADIVVFDEISMATNYDLSVVNARLRAKHYVYI
GDPAQLPAPRTLLTKGTLEPEYFNSVCRLMKTIGPDMFLGTCRRC
PAEIVDTVSALVYDNKLKAHKDKSAQCFKMFYKGVITHDVSSAIN
RPQIGVVREFLTRNPAWRKAVFISPYNSQNAVASKILGLPTQTVD
SSQGSEYDYVIFTQTTETAHSCNVNRFNVAITRAKVGILCIMSDR
DLYDKLQFTSLEIPRRNVATLQ**

Native sequence Amino acids A1 – Q601 (end).
Residue A232 of the fusion protein is equivalent to A1 of the native enzyme. The GST tag is located at residues 1 – 220.

Protease cleavage PreScission (LEVLFQGP) residues 221 – 228