SI9: Nsp13

Table 1: General Information

1	**Protein Name (according to NCBI Reference Sequence NC_045512.2)**
	ORF1ab; nsp13
2	**Region/Name/Further Specification**
	NTPase / helicase domain / RNA 5'-triphosphatase
3	**Sequence of fl protein (according to NCBI Reference Sequence NC_045512.2)**
	AVGACVLCNSQTSLRCGACIRRPFLCCKCCYDHVISTSHKLVLSVNPYVCNAPGCDVTDVTQL YLGGMSYYCKSHKPPISFPLCANGQVFGLYKNTCVGSDNVTDFNAIATCDWTNAGDYILANTC TERLKLFAAETLKATEETFKLSYGIATVREVLSDRELHLSWEVGKPRPPLNRNYVFTGYRVTKN SKVQIGEYTFEKGDYGDAVVYRGTTTYKLNVGDYFVLTSHTVMPLSAPTLVPQEHYVRITGLY PTLNISDEFSSNVANYQKVGMQKYSTLQGPPGTGKSHFAIGLALYYPSARIVYTACSHAAVDAL CEKALKYLPIDKCSRIIPARARVECFDKFKVNSTLEQYVFCTVNALPETTADIVVFDEISMATNY DLSVVNARLRAKHYVYIGDPAQLPAPRTLLTKGTLEPEYFNSVCRLMKTIGPDMFLGTCRRCPA EIVDTVSALVYDNKLKAHKDKSAQCFKMFYKGVITHDVSSAINRPQIGVVREFLTRNPAWRKA VFISPYNSQNAVASKILGLPTQTVDSSQGSEYDYVIFTQTTETAHSCNVNRFNVAITRAKVGILCI MSDRDLYDKLQFTSLEIPRRNVATLQ
4	**Protein boundaries of expressed construct (according to NCBI Reference Sequence NC_045512.2)**
	1-601 aa (fl nsp13)
5	**Ratio for construct design**
	fl protein
6	**Sequence homology (to SCoV)**
	Identity: 99.8%; similarity: 100%
7	**Published structures (SCoV2 or homologue variants)**
	SCoV2: PDB 6ZSL, 6JYT, 6XEZ
8	**(Published) assignment (SCoV2 or homologue variants)**
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Table 2: Protein Expression

1	**Expression vector**
	pE-SUMO (LifeSensors)
2	**Purification-/Solubility-Tag**
	N-terminal His_6-SUMO
3	**Cleavage Site**
	Ulp1
4	**Molecular weight / Extinction coefficient / pI - of cleaved protein**

	66.85 kDa / 67,160 M^{-1} cm^{-1} / 8.66
5	**Comments on sequence of expressed construct**
	No artificial residues due to Ulp1-cleavage and construct design.
6	**Used expression strain**
	E. coli BL21 (DE3)
7	**Cultivation medium**
	LB / M9 (uniformly ^{15}N-labelled)
8	**Induction system**
	IPTG inducible T7 promoter
9	**Induction of protein expression**
	0.2 mM IPTG at OD_{600} 0.6-0.7 (addition of 50 µM $ZnCl_2$)
10	**Cultivation temperature and time**
	18-20°C for 16-18 h

Table 3: Protein Purification

1	**Buffer List**
A	25 mM Tris (pH 8.0), 300 mM NaCl, 5 mM imidazole, 5% (v/v) glycerol, 10 mM bME (cell disruption / IMAC).
B	20 mM BisTris (pH 7.0), 150 mM NaCl, 2 mM TCEP-HCl (SEC/ final NMR buffer).
2	**Purification steps (with corresponding buffer(s) and incubation times)**
A	Cell disruption in buffer **1A** (plus one tablet of EDTA free protease inhibitor cocktail (Merck) and addition of 50 µM $ZnCl_2$) by microfluidization.
B	IMAC (HisTrap HP (GE Healthcare), ÄKTA start (GE Healthcare)), elution with imidazole gradient up to 500 mM in buffer **1A**.
C	SEC (HiLoad 26/600 SD 200 pg (GE Healthcare), ÄKTApurifier (GE Healthcare)) in buffer **1B** (elution volume 210-240 mL).
D	NMR sample preparation in buffer **1B.**

Table 4: Final sample

1	**Yield**
	0.5 mg/L ^{15}N-M9 medium
2	**Stability**
	Aggregation at > 20 µM under these conditions.
3	**Comment on applicability**
	Not suitable for NMR experiments.

Additional information

Constructs	**Conditions**	**Comments**
aa 1-601 (fl nsp13); His_6 (pET28a(+) (GenScript)), TEV-cleavage site, N-terminal “GHM“ three artificial residues.	Native (as above)	Weak expression, instable protein.

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