SI1: Nsp1

Table 1: General Information

1	**Protein Name (according to NCBI Reference Sequence NC_045512.2)**
	ORF1a and ORF1ab; nsp1
2	**Region/Name/Further Specification**
	Nsp1 / Leader protein
3	**Sequence of fl protein (according to NCBI Reference Sequence NC_045512.2)**
	MESLVPGFNEKTHVQLSLPVLQVRDVLVRGFGDSVEEVLSEARQHLKDGTCGLVEVEKGVLPQ LEQPYVFIKRSDARTAPHGHVMVELVAELEGIQYGRSGETLGVLVPHVGEIPVAYRKVLLRKNG NKGAGGHSYGADLKSFDLGDELGTDPYEDFQENWNTKHSSGVTRELMRELNGG
4	**Protein boundaries of expressed construct (according to NCBI Reference Sequence NC_045512.2)**
fl	aa 1-180 (fl nsp1)
GD	aa 13-127 of fl nsp1
5	**Ratio for construct design**
fl	fl sequence according to NCBI Reference Sequence YP_009725297.1
GD	In analogy to the available NMR structure (PDB 2GDT) of nsp1 SCoV 13-127
6	**Sequence homology (to SCoV)**
fl	Identity: 83%; similarity: 89%
GD	Identity: 85%; similarity: 90%
7	**Published structures (SCoV2 or homologue variants)**
	SCoV: PBD 2GDT, 2HSX SCoV2: PBD 7K3N, 7K7P, 6ZN5, 7JQC, 7K5I
8	**(Published) assignment (SCoV2 or homologue variants)**
	SCoV: BMRB 7014 SCoV2: BMRB 50620

Table 2: Protein Expression

1	**Expression vector**
fl	pETM11 (Gunter Stier, EMBL Heidelberg)
GD	pKM263 (GenScript)
2	**Purification-/Solubility-Tag**
fl	N-terminal His_6
GD	N-terminal His_6
3	**Cleavage Site**
	TEV
4	**Molecular weight / Extinction coefficient / pI - of cleaved protein**

fl	19.90 kDa / 12,950 M^{-1} cm^{-1} / 5.37
GD	12.93 kDa / 4,470 M^{-1} cm^{-1} / 6.22
5	**Comments on sequence of expressed construct**
fl	N-terminal „GA" two artificial residues due to TEV-cleavage and construct design
GD	N-terminal „GAMA" four artificial residues due to TEV-cleavage and construct design
6	**Used expression strain**
	E. coli BL21 (DE3)
7	**Cultivation medium**
	LB / M9 (uniformly ^{15}N or ^{13}C,^{15}N-labelled)
8	**Induction system**
	IPTG inducible T7 promoter
9	**Induction of protein expression**
fl	0.6 mM IPTG at OD_{600} 0.7
GD	1 mM IPTG
10	**Cultivation temperature and time**
fl	16°C for 18-20 h
GD	16°C for 18-20 h

Table 3a: Protein Purification (fl nsp1)

1	**Buffer List**
A	50 mM Tris-HCl (pH 7.5), 500 mM NaCl, 100 mM Na_2SO_4, 5% (v/v) glycerol, 5 mM imidazole, 1 mM TCEP-HCl (cell disruption / IMAC / TEV-cleavage).
B	50 mM Tris-HCl (pH 7.5), 500 mM NaCl, 100 mM Na_2SO_4, 1 mM EDTA, 1 mM TCEP-HCl (SEC).
C	50 mM NaPi (pH 6.5), 200 mM NaCl, 2 mM DTT, 2 mM EDTA (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 (Roche), 100 µg of lysozyme (Carl Roth), and 50 µg of deoxyribonuclease (DNAse) (New England Biolabs)) by sonication.
B	IMAC (gravity flow Ni^{2+}-NTA (Cytiva)), washed first with buffer **1A** and then with buffer **1A** containing additional 2 M LiCl, before eluting with 300 mM imidazole in buffer **1A**.
C	Desalting and TEV-cleavage (0.5 mg TEV protease per 1 L culture) o.n. in buffer **1A**.
D	SEC on HiLoad 16/600 SD 75 (GE Healthcare) in buffer **1B**.
E	NMR sample preparation in buffer **1C**.

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Table 3b: Protein Purification (GD nsp1)

1	**Buffer List**
A	50 mM Tris-HCl (pH 8.0), 300 mM NaCl, 10 mM imidazole, 4 mM DTT (cell disruption / IMAC/ dialysis after IMAC / TEV-cleavage).
B	25 mM NaPi (pH 7.0), 250 mM NaCl, 2 mM TCEP-HCl, 0.02% (w/v) NaN_3 (SEC / final NMR buffer).
2	**Purification steps (with corresponding buffer(s) and incubation times)**
A	Cell disruption in buffer **1A** (plus 100 µL protease inhibitor (Serva)) by sonication.
B	IMAC (gravity flow Ni^{2+}-NTA), Elution with 150-500 mM imidazole in buffer **1A**.
C	Dialysis o.n. in in buffer **1A**.
D	TEV-cleavage (0.5 mg TEV protease per 1 L culture) in buffer **1A**.
E	SEC on HiLoad 16/600SD 75 (GE Healthcare) in buffer **1B**.
F	NMR sample preparation in buffer **1B**.

Table 4: Final samples

1	**Yield**
fl	5 mg/L ^{13}C,^{15}N-M9 medium
GD	< 0.5 mg/L ^{5}N-M9 medium
2	**Stability**
fl	No significant precipitation or degradation observed after storage at 4°C for 3 weeks. Relatively stable during NMR measurements at 25°C for ~7 days, despite some proteolysis of disordered C-terminal tail.
GD	Stable during several weeks storage at 4°C.
3	**Comment on applicability**
fl	Suitable for NMR structure determination, fragment screening, interaction studies.
GD	Purification needs optimization to obtain more soluble protein

Additional information

Constructs	**Conditions**	**Comments**
aa 1-180 (fl nsp1); His_7 (pET-TEV-Nco (GenScript)), TEV-cleavage site, N-terminal 2 artificial residues "GA".	As above for GD nsp1.	Yields 2.4 mg/L ^{15}N,^{13}C-M9 medium. Obvious degradation during measurement. Storage at 4°C not advisable. Higher salt concentration seems to slightly improve stability.

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