SI6: Nsp8

Table 1: General Information

1	**Protein Name (according to NCBI Reference Sequence NC_045512.2)**
	ORF1a and ORF1ab; nsp8
2	**Region/Name/Further Specification**
	Nsp8
3	**Sequence of fl protein (according to NCBI Reference Sequence NC_045512.2)**
	AIASEFSSLPSYAAFATAQEAYEQAVANGDSEVVLKKLKKSLNVAKSEFDRDAAMQRKLEKM ADQAMTQMYKQARSEDKRAKVTSAMQTMLFTMLRKLDNDALNNIINNARDGCVPLNIIPLTT AAKLMVVIPDYNTYKNTCDGTTFTYASALWEIQQVVDADSKIVQLSEISMDNSPNLAWPLIVT ALRANSAVKLQ
4	**Protein boundaries of expressed construct (according to NCBI Reference Sequence NC_045512.2)**
	aa 1-198 (fl nsp8)
5	**Ratio for construct design**
	fl protein
6	**Sequence homology (to SCoV)**
	Identity: 97%; similarity: 98%
7	**Published structures (SCoV2 or homologue variants)**
	SCoV: PDB 6NUS, 6NUR, 2AHM, SCoV2: PDB 7C2K, 7BV2, 7BV1, 7CTT, 6M5I, 7BW4, 6XEZ, 7BZF, 6XQB, 6M71, 6YYT, 7BTF, 7JLT, 7AAP, 6WIQ, 6XIP, 6WQD, 6WTC, 6YHU
8	**(Published) assignment (SCoV2 or homologue variants)**
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Table 2: Protein Expression

1	**Expression vector**
	pET46
2	**Purification-/Solubility-Tag**
	N-terminal His_6, enterokinase
3	**Cleavage Site**
	TEV
4	**Molecular weight / Extinction coefficient / pI - of cleaved protein**
	21.94 kDa / 19,940 $cm^{-1}M^{-1}$ / 6.5
5	**Comments on sequence of expressed construct**
	N-terminal “G**”** an artificial residue due to TEV-cleavage.

 doi:10.3389/fmolb.2021.653148

6	**Used expression strain**
	E. coli Rosetta2 pLysS
7	**Cultivation medium**
	M9 (uniformly ^{15}N-, ^{13}C-labelled)
8	**Induction system**
	IPTG inducible T7 promoter
9	**Induction of protein expression**
	0.5 mM IPTG at OD_{600} 0.8
10	**Cultivation temperature and time**
	16˚C for 16-18 h

Table 3: Protein Purification

1	**Buffer List**
A	10 mM HEPES (pH 7.4), 300 mM NaCl, 30 mM Imidazole, 2 mM DTT.
B	10 mM HEPES (pH 7.4), 300 mM NaCl, 300 mM Imidazole, 2 mM DTT.
C	10 mM MOPS (pH 7.0), 300 mM NaCl, 2 mM DTT.
D	10 mM MOPS (pH 7.0), 150 mM NaCl, 2 mM DTT.
2	**Purification steps (with corresponding buffer(s) and incubation times)**
A	Cell lysis in buffer **1A** by microfluidizer operating at 20,000 psi. Lysates were cleared by centrifugation at 25,000 g for 30 min and then filtered through a 0.45 µm filter. Ni-NTA Agarose beads (Qiagen) were added to cleared lysates and incubated for 30 min. Beads were collected by centrifugation and then loaded onto a gravity column. Beads were washed twice with 10 column volumes of buffer **1A**. Protein was eluted with 5 column volumes of buffer **1B.**
B	Eluted protein was cleaved with 1% (w/w) TEV protease o.n. at rt while dialyzing the protein into 1L buffer **1C**. Uncleaved protein was removed by inverse Ni-NTA binding.
C	Protein was concentrated using a 10 kDa MWCO (Amicon) concentrator and purified on aSD 200 Increase 10/300 (GE Life Sciences) size exclusion column, AKTApure (GE Life Sciences) using buffer **1D**.

Table 4: Final sample

1	**Yield**
	17 mg/L ^{13}C,^{15}N-M9 medium
1b	**A260/280 ratio**
	0.5
2	**Stability**
	Concentration dependent aggregation of nsp8 observed in the range of 0.1-1.1mM by NMR.

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3	**Comment on applicability**
	Suitable for NMR-based screening approach.

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