SI3: Nsp3e

Table 1: General Information

1	**Protein Name (according to NCBI Reference Sequence NC_045512.2)**
	ORF1a and ORF1ab; nsp3
2	**Region/Name/Further Specification**
	nsp3e / NAB globular domain
3	**Sequence of "fl" protein (aa 1080-1203 of complete Nsp3, according to NCBI Reference Sequence NC_045512.2)**
	YFTEQPIDLVPNQPYPNASFDNFKFVCDNIKFADDLNQLTGYKKPASRELKVTFFPDLNGDVVA IDYKHYTPSFKKGAKLLHKPIVWHVNNATNKATYKPNTWCIRCLWSTKPVET
4	**Protein boundaries of expressed construct (according to NCBI Reference Sequence NC_045512.2)**
	aa 1,088-1,203 of complete nsp3
5	**Ratio for construct design**
	Based on boundaries from NMR structure of homologue nsp3e from SARS-CoV (2K87).
6	**Sequence homology (to SCoV)**
	Identity: 82%; similarity: 89%
7	**Published structures (SCoV2 or homologue variants)**
	SCoV: PDB 2K87
8	**(Published) assignment (SCoV2 or homologue variants)**
	SCoV: BMRB 15723; SCoV2: BMRB 50334

Table 2: Protein Expression

1	**Expression vector**
	pKM263 (GenScript)
2	**Purification-/Solubility-Tag**
	N-terminal His_6-GST
3	**Cleavage Site**
	TEV
4	**Molecular weight / Extinction coefficient / pI - of cleaved protein**
	13.75 kDa / 25,565 M^{-1} cm^{-1} / 8.9
5	**Comments on sequence of expressed construct**
	N-terminal „GAMG" four artificial residues due to TEV-cleavage and construct design.
6	**Used expression strain**

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	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**
	1 mM IPTG at OD_{600} 0.7
10	**Cultivation temperature and time**
	20-22°C for 18-20 h

Table 3: Protein Purification

1	**Buffer List**
A	50 mM NaPi (pH 6.5), 300mM NaCl, 10 mM imidazole, 2 mM TCEP-HCl (cell disruption / IMAC).
B	25 mM NaPi (pH 7.0), 150 mM NaCl, 2 mM DTT, 0.02% (w/v) NaN_3 (dialysis after IMAC / TEV-cleavage).
C	25 mM NaPi (pH 7.0), 150 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) (Carl Roth, Germany), elution with 150-500 mM imidazole in buffer **1A**.
C	Dialysis o.n. in buffer **1B**.
D	TEV-cleavage (0.5 mg TEV protease per 1 L culture) in buffer **1B**.
E	SEC on HiLoad 16/600SD 75 (GE Healthcare) in buffer **1C**.
F	NMR sample preparation in buffer **1C**.

Table 4: Final sample

1	**Yield**
	3.5 mg/L ^{13}C, ^{15}N-M9 medium
2	**A260/280 ratio**
	0.74
3	**Stability**
	Stable throughout measurement (7 days, 298 K). No significant precipitation or degradation observed after storage at 4°C for 5 weeks.
4	**Comment on applicability**

	Suitable for NMR structure determination, fragment screening, interaction studies.

Additional information

Constructs	**Conditions**	**Comments**
NAB (aa 1,088-1,203) of complete nsp3; His_7 (pET-TEV-Nco (GenScript)), TEV-cleavage site, N-terminal “GAMG“ four artificial residues.	As above.	Works as well, but slightly less expression and yield.

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