SI17: ORF7a

Table 1: General Information

1	**Protein Name (according to NCBI Reference Sequence NC_045512.2)**
	ORF7a
2	**Region/Name/Further Specification**
	Ectodomain (ED)
3	**Sequence of fl protein (according to NCBI Reference Sequence NC_045512.2)**
	MKIILFLALITLATCELYHYQECVRGTTVLLKEPCSSGTYEGNSPFHPLADNKFALTCFSTQFAFA CPDGVKHVYQLRARSVSPKLFIRQEEVQELYSPIFLIVAAIVFITLCFTLKRKTE
4	**Protein boundaries of expressed construct (according to NCBI Reference Sequence NC_045512.2)**
	aa 16-81 (ectodomain of ORF7a)
5	**Ratio for construct design (detailed and comprehensible)**
	Only the ectodomain without signaling peptide. Transmembrane helix is also not included in the construct.
6	**Sequence homology (to SCoV)**
	Identity: 85.3%; similarity: 95.9%
7	**Published structures (SCoV2 or homologue variants)**
	SCoV: PDB 1XAK, 1YO4
8	**(Published) assignment (SCoV2 or homologue variants)**
	SCoV: BMRB 6824

Table 2: Protein Expression

1	**Expression vector**
	pET24d-GB1 (Novagen, modified by G. Stier (Bogomolovas et al., 2009))
2	**Purification-/Solubility-Tag**
	N-terminal His_6-GB1
3	**Cleavage Site**
	TEV
4	**Molecular weight / Extinction coefficient / pI - of cleaved protein**
	7.49 kDa / 6,210 M^{-1} cm^{-1} / 6.99
5	**Comments on sequence of expressed construct**
	N-terminal „G" one artificial residue due to TEV-cleavage and construct design.
6	**Used expression strain**

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	E.coli (DE3) BL21
7	**Cultivation medium**
	M9 (uniformly ^{15}N-labelled)
8	**Induction system**
	IPTG inducible T7 promoter
9	**Induction of protein expression**
	0.2 mM IPTG at OD_{600} 0.7
10	**Cultivation temperature and time**
	25°C for 18-20 h

Table 3: Protein Purification

1	**Buffer List**
A	20 mM Tris-HCl (pH 8.0), 6 M GdnHCl, 500 mM NaCl, 5 mM imidazole, 2 mM bME (Cell disruption / solubilization of pellet).
B	20 mM Tris (pH 8.0), 6 M GdnHCl, 500 mM NaCl, 10 mM imidazole, 2 mM bME (IMAC1).
C	50 mM NaPi (pH 8.0), 300 mM NaCl, 10 mM imidazole, 2 mM bME (IMAC2).
D	1 mM acetate-D4 (pH 5.0) (final NMR-buffer).
2	**Purification steps (with corresponding buffer(s) and incubation times)**
A	Cell disruption and solubilization of pellet in buffer **1A**.
B	IMAC, gravity flow Ni^{2+}-NTA (Qiagen), elution with 200 mM imidazole in buffer **1B**.
C	Dialysis against buffer **1C**.
D	TEV-cleavage (1 mg TEV protease per 10 mL protein solution) o.n. in buffer **1C**.
E	Inv. IMAC, elution with 200 mM imidazole in buffer **1C**.
F	Dialysis of flow-through of inv. IMAC against **1D** and concentrate (NMR-sample).

Table 4: Final sample

1	**Yield**
	0.4 mg/L ^{15}N-M9 medium
1b	**A260/280 ratio**
	0.7
2	**Stability**
	Stable throughout measurement (1 day, 298/315 K). No precipitation or degradation observed after four days at rt.

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3	**Comment on applicability**
	Suitable for NMR structure determination, fragment screening, interaction studies.