SI11: Nsp15

Table 1: General Information

1	**Protein Name (according to NCBI Reference Sequence NC_045512.2)**
	ORF1ab; nsp15
2	**Region/Name/Further Specification**
	Nsp15 / NendoU / Endonucleasee
3	**Sequence of fl protein (according to NCBI Reference Sequence NC_045512.2)**
	SLENVAFNVVNKGHFDGQQGEVPVSIINNTVYTKVDGVDVELFENKTTLPVNVAFELWAKRNI KPVPEVKILNNLGVDIAANTVIWDYKRDAPAHISTIGVCSMTDIAKKPTETICAPLTVFFDGRVD GQVDLFRNARNGVLITEGSVKGLQPSVGPKQASLNGVTLIGEAVKTQFNYYKKVDGVVQQLPE TYFTQSRNLQEFKPRSQMEIDFLELAMDEFIERYKLEGYAFEHIVYGDFSHSQLGGLHLLIGLAK RFKESPFELEDFIPMDSTVKNYFITDAQTGSSKCVCSVIDLLLDDFVEIIKSQDLSVVSKVVKVTI DYTEISFMLWCKDGHVETFYPKLQ
4	**Protein boundaries of expressed construct (according to NCBI Reference Sequence NC_045512.2)**
	aa 1-346 (fl nsp15)
5	**Ratio for construct design**
	fl protein
6	**Sequence homology (to SCoV)**
	Identity: 89%; similarity: 98%
7	**Published structures (SCoV2 or homologue variants)**
	SCoV: PDB 2H85 SCoV2: PDB 6W01
8	**(Published) assignment (SCoV2 or homologue variants)**
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Table 2: Protein Expression

1	**Expression vector**
	pET28a(+) (GenScript)
2	**Purification-/Solubility-Tag**
	N-terminal His_6
3	**Cleavage Site**
	TEV
4	**Molecular weight / Extinction coefficient / pI - of cleaved protein**
	39.14 kDa / 32,890 M^{-1} cm^{-1} / 5.12
5	**Comments on sequence of expressed construct**

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	N-terminal “GHM” three 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-labelled)
8	**Induction system**
	IPTG inducible T7 promoter
9	**Induction of protein expression**
	0.2 mM IPTG at OD_{600} 0.6-0.7
10	**Cultivation temperature and time**
	18-20°C for 16-18 h

Table 3: Protein Purification

1	**Buffer List**
A	25 mM Tris-HCl (pH 8.0), 300 mM NaCl, 5 mM imidazole, 5% (v/v) glycerol, 10 mM bME (cell disruption / IMAC).
B	25 mM NaPi (pH 7.5), 300 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)) 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 200-260 mL).
D	NMR sample preparation in buffer **1B**.

Table 4: Final sample

1	**Yield**
	5 mg/L ^{15}N-M9 medium
2	**Stability**
	Tendency to aggregate at rt.
3	**Comment on applicability**
	Suitable for fragment screening and interaction studies.

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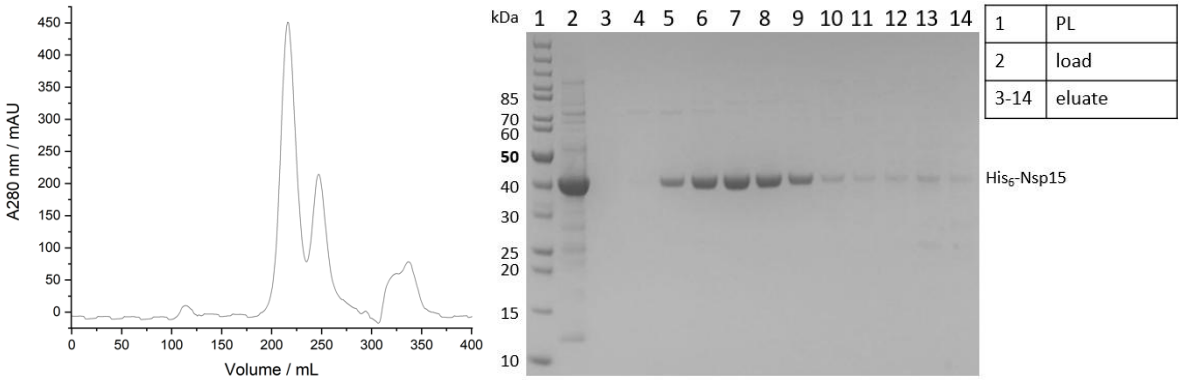

Analytical SEC of nsp15. Protein was eluted from 200-260 mL (left panel) with corresponding SDS-PAGE of SEC with fractions analyzed from 190-260 mL (right panel).

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