SI3: Nsp3b

Table 1: General Information

1	**Protein Name (according to NCBI Reference Sequence NC_045512.2)**
	ORF1a and ORF1ab; nsp3
2	**Region/Name/Further Specification**
	Nsp3b / Macrodomain
3	**Sequence of "fl" protein (aa 207-376 of complete nsp3, according to NCBI Reference Sequence NC_045512.2)**
	VNSFSGYLKLTDNVYIKNADIVEEAKKVKPTVVVNAANVYLKHGGGVAGALNKATNNAMQV ESDDYIATNGPLKVGGSCVLSGHNLAKHCLHVVGPNVNKGEDIQLLKSAYENFNQHEVLLAPL LSAGIFGADPIHSLRVCVDTVRTNVYLAVFDKNLYDKLVSSFLEMK
4	**Protein boundaries of expressed construct (according to NCBI Reference Sequence NC_045512.2)**
	aa 207-376 of complete nsp3
5	**Ratio for construct design**
	Based on homologous structure from SCoV (PDB 6VXS).
6	**Sequence homology (to SCoV)**
	Identity: 74%; similarity: 84%
7	**Published structures (SCoV2 or homologue variants)**
	SCoV2: PDB 6W6Y, 6YWM, 6YWL, 6YWK, 6WEY, 7KG3, 6W02, 6WOJ, 6WEN, 6WCF, 6VXS, 7JME
8	**(Published) assignment (SCoV2 or homologue variants)**
	SCoV2: BMRB 50387 (apo), 50388 (holo)

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**
	18.65 kDa / 10,430 M^{-1} cm^{-1} / 7.20
5	**Comments on sequence of expressed construct**
	N-terminal "GHM" three artificial residues due to TEV-cleavage and construct design.

6	**Used expression strain**
	E. coli T7 Express
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**
	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, 10 mM bME (cell disruption / IMAC).
B	25 mM Tris-HCl (pH 8.0), 300 mM NaCl, 10 mM bME (dialysis after IMAC / TEV-cleavage).
C	25 mM BisTris (pH 6.5), 150 mM NaCl, 3 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	TEV-cleavage (1 mg TEV protease per 50 mL protein solution) o.n. in buffer **1B.**
D	Inv. IMAC (HisTrap HP (GE Healthcare), ÄKTA start (GE Healthcare)), elution with 500 mM imidazole in buffer **1A**.
E	SEC (HiLoad 26/600 SD 200 pg (GE Healthcare), ÄKTApurifier (GE Healthcare)) in buffer **1C** (elution volume 245-290 mL).
F	NMR sample preparation in buffer **1C.**

Table 4: Final sample

1	**Yield**
	94 mg/L ^{15}N-M9 medium, 9 mg/L ^{13}C,^{15}N-M9 medium
2	**Stability**
	Stable throughout measurement (7 days, 298 K). No significant precipitation or degradation observed after storage at 4°C for 2 weeks.
3	**Comment on applicability**

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

Additional information

Constructs	**Conditions**	**Comments**
aa 206-374 of complete nsp3; His_6-GST (mod pET9d), TEV-cleavage site, N-terminal "GAM" three artificial residues. Based on boundaries from crystal structure (PDB 6W6Y).	**IMAC buffer:** 50 mM Tris-HCl (pH 8.0), 500 mM NaCl, 5% (v/v) glycerol, 50 mM Imidazole, 1 mM DTT. **Cleavage buffer:** 50 mM Tris-HCl (pH 8.0), 500 mM NaCl, 1 mM DTT. **SEC/final buffer:** 20 mM NaPi (pH 7.4), 150 mM NaCl, 3 mM TCEP-HCl.	Yields 30 mg/L LB medium. No significant precipitation or degradation observed after storage at 4°C for 10 days. Suitable for NMR studies, fragment-based screening, interaction studies.

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