

SI8: Nsp10

Table 1: General Information

1	Protein Name (according to NCBI Reference Sequence NC_045512.2)
	ORF1a and ORF1ab; nsp10
2	Region/Name/Further Specification
	Nsp10
3	Sequence of fl protein (according to NCBI Reference Sequence NC_045512.2)
	AGNATEVPANSTVLSFCAFAVDAAKAYKDYLASGGQPITNCVKMLCTHTGTGQAITVTPEAN MDQESFGGASCCLYCRCHIDHPNPKGFCDLKGKYVQIPTTCANDPVGFTLKNTVCTVCGMWK GYGCSCDQLREPLQ
4	Protein boundaries of expressed construct (according to NCBI Reference Sequence NC_045512.2)
	aa 1-139 (fl nsp10)
5	Ratio for construct design
	fl protein
6	Sequence homology (to SCoV)
	Identity: 97%; similarity: 99%
7	Published structures (SCoV2 or homologue variants)
	SCoV: PDB 5C8S, 5NFY, 2FYG, 2XYQ, 2XYV, 2XYW SCoV2: PDB 6W4H, 6W61, 7JYY, 7C2I, 7BQ7, 2G9T
8	(Published) assignment (SCoV2 or homologue variants)
	SCoV2: BMRB 50392

Table 2: Protein Expression

1	Expression vector
	pET21b(+) (GenScript)
2	Purification-/Solubility-Tag
	N-terminal His ₆
3	Cleavage Site
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4	Molecular weight / Extinction coefficient / pI - of protein
	16.24 kDa / 12,950 M ⁻¹ cm ⁻¹ / 6.72
5	Comments on sequence of expressed construct
	N-terminal "MGSDKIHHHHHH" twelve artificial residues due to construct design
6	Used expression strain

	<i>E. coli</i> 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
	0.5 mM IPTG at OD ₆₀₀ 0.6-0.7 (addition of 50 μM ZnCl ₂)
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	50 mM NaPi (pH 7.5), 50 mM NaCl, 5 mM DTT (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) and addition of 50 μM ZnCl ₂) 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 75 μg (GE Healthcare), ÄKTApurifier (GE Healthcare)) in buffer 1B (elution volume 175-225 mL).
D	NMR sample preparation in buffer 1B .

Table 4: Final sample

1	Yield
	25 mg/L ¹⁵ N-M9 medium, 15 mg/L ¹³ C, ¹⁵ N-M9 medium
2	Stability
	Stable throughout measurement (6 days, 298 K). No significant precipitation or degradation observed after storage at -80°C for 2 months.
3	Comment on applicability
	Suitable for NMR structure determination, fragment screening, interaction studies.

Additional information

Constructs	Conditions	Comments
aa 1-139 (fl nsp10); His ₆ (pMCSG53 (BEI Resources, cat.	IMAC-buffer: 50 mM Tris-HCl (pH 9.0), 0.5 M NaCl, 10 mM bME,	Yields 30-40 mg/L 2xTY medium. Can be flash-frozen in liquid

NR-52425)), TEV cleavage site, N-terminal “SNM” three artificial residues.	2 mM MgCl ₂ , 0.1% (v/v) Triton X-100, 5-10% (v/v) glycerol, 50 mM imidazole. SEC-buffer: 20 mM HEPES (pH 8.5), 0.5 M NaCl, 10 mM bME, 2 mM MgCl ₂ , 5% (v/v) glycerol, 20 mM imidazole.	nitrogen and stored at 20°C, used for nsp14 and nsp16 stabilization at 1:1 molar ratios.
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