SI3: Nsp3d

Table 1: General Information

1	**Protein Name (according to NCBI Reference Sequence NC_045512.2)**
	ORF1a and ORF1ab; nsp3
2	**Region/Name/Further Specification**
	Nsp3d / papain-like protease / PL^{pro}
3	**Sequence of "fl" protein (aa 743-1060 of complete nsp3, according to NCBI Reference Sequence NC_045512.2)**
	SLREVRTIKVFTTVDNINLHTQVVDMSMTYGQQFGPTYLDGADVTKIKPHNSHEGKTFYVLPN DDTLRVEAFEYYHTTDPSFLGRYMSALNHTKKWKYPQVNGLTSIKWADNNCYLATALLTLQQ IELKFNPPALQDAYYRARAGEAANFCALILAYCNKTVGELGDVRETMSYLFQHANLDSCKRVL NVVCKTCGQQQTTLKGVEAVMYMGTLSYEQFKKGVQIPCTCGKQATKYLVQQESPFVMMSA PPAQYELKHGTFTCASEYTGNYQCGHYKHITSKETLYCIDGALLTKSSEYKGPITDVFYKENSY TTTIK
4	**Protein boundaries of expressed construct (according to NCBI Reference Sequence NC_045512.2)**
	aa 743-1,060 of complete nsp3
5	**Ratio for construct design**
	Based on homologous structure from SCoV (PDB 4M0W)
6	**Sequence homology (to SCoV)**
	Identity: 83%; similarity: 91%
7	**Published structures (SCoV2 or homologue variants)**
	SCoV: PDB 4M0W, 2FE8 SCoV2: PDB 6W9C
8	**(Published) assignment (SCoV2 or homologue variants)**
	-

Table 2: Protein Expression

1	**Expression vector**
	pE-SUMO (LifeSensors)
2	**Purification-/Solubility-Tag**
	N-terminal His_6-SUMO
3	**Cleavage Site**
	Ulp1
4	**Molecular weight / Extinction coefficient / pI - of cleaved protein**
	35.99 kDa / 45,270 M^{-1} cm^{-1} / 8.17
5	**Comments on sequence of expressed construct**

 doi:10.3389/fmolb.2021.653148

	No artificial residues due to Ulp1-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 (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	20 mM Tris-HCl (pH 8.0), 300 mM NaCl, 10 mM imidazole, 50 µM $ZnCl_2$, 10 mM bME (cell disruption / IMAC).
B	10 mM HEPES (pH 7.4), 100 mM NaCl, 50 µM $ZnCl_2$, 10 mM bME (dialysis after IMAC / TEV-cleavage).
C	10 mM HEPES (pH 7.4), 100 mM NaCl, 50 µM $ZnCl_2$, 5 mM DTT (SEC).
2	**Purification steps (with corresponding buffer(s) and incubation times)**
A	Cell disruption in buffer **1A** (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	Ulp1-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 75 pg (GE Healthcare), ÄKTApurifier (GE Healthcare)) in buffer **1C** (elution volume 180-220 mL).

Table 4: Final sample

1	**Yield**
	12 mg/L ^{15}N-M9 medium
2	**Stability**
	Tendency to aggregate.
3	**Comment on applicability**

	Suitable for fragment screening, interaction studies.

Additional information

Constructs	**Conditions**	**Comments**
aa 743-1,060 of complete nsp3; His_6 (pET28a(+) (GenScript)), TEV-cleavage site, N-terminal "GHM" three artificial residues.	Native (as above)	Weak expression, less protein.

 doi:10.3389/fmolb.2021.653148