SI22: ORF14

Table 1: General Information

1	**Protein Name**
	ORF14
2	**Region/Name/Further Specification**
3	**Sequence of fl protein**
	MLQSCYNFLKEQHCQKASTQKGAEAAVKPLLVPHHVVATVQEIQLQAAVGELLLLEWLAMA VMLLLLCCCLTD
4	**Protein boundaries of expressed construct**
	aa 1-73 (fl ORF14)
5	**Ratio for construct design**
	fl protein
6	**Sequence homology (to SCoV)**
	Identity: NA; similarity: NA
7	**Published structures (SCoV2 or homologue variants)**
	-
8	**(Published) assignment (SCoV2 or homologue variants)**
	-

Table 2: Cell-free Protein Synthesis

1	**Expression vector**
	pEU-E01-MCS (Cell-Free Sciences)
2	**Purification-/Solubility-Tag**
	N-terminal Strep tag II (WSHPQFEK)
3	**Cleavage Site**
	-
4	**Molecular weight / Extinction coefficient / pI - of protein**
	9.26 kDa / 12,490 M^{-1} cm^{-1} / 6.01
5	**Comments on sequence of expressed construct**
	N-terminal “WSHPQFEKGGG” eleven artificial residues due to construct design.
6	**Feeding buffer**

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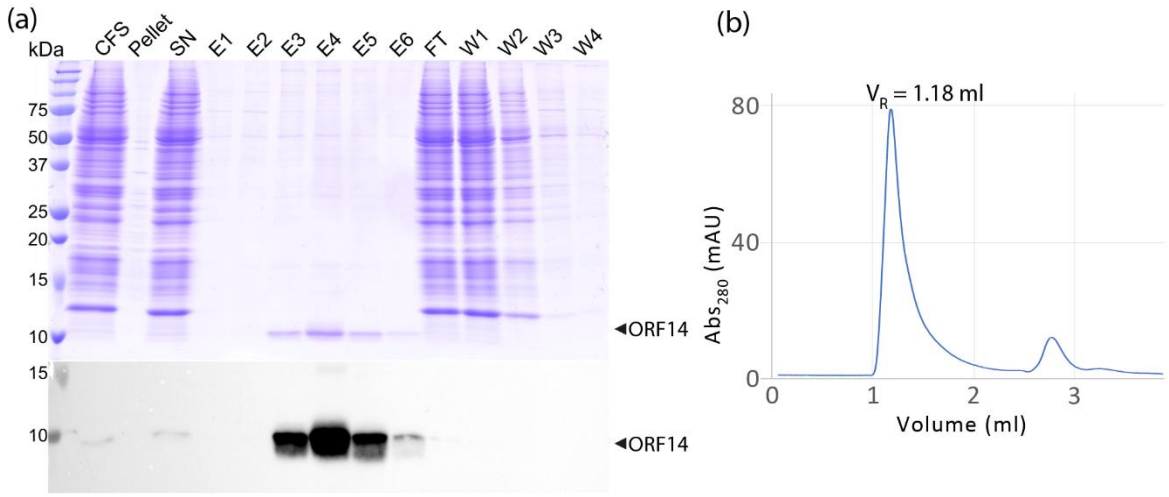
	30 mM HEPES-KOH (pH 7.6), 100 mM potassium acetate, 2.7 mM magnesium acetate, 16 mM creatine phosphate, 0.4 mM spermidine, 1.2 mM ATP, 0.25 mM GTP, 4 mM DTT and 6 mM (average concentration) amino acid mix and 0.05% (w/v) Brij-58.
7	**Translation mix**
	50% (v/v) mRNA, 50% (v/v) home-made WGE, 40 μg/mL creatine kinase, and 6 mM (average concentration) amino acid mix 0.05% (w/v) Brij-58.
8	**Protein synthesis temperature and time**
	22°C for 16 h without agitation (bilayer method).

Table 3: Protein Purification

1	**Buffer List**
A	100 mM Tris-HCl (pH 8.0), 150 mM NaCl, 1 mM EDTA, 0.1% (w/v) DDM (wash buffer).
B	100 mM Tris-HCl (pH 8.0), 150 mM NaCl, 1 mM EDTA, 2.5 mM desthiobiotin, and 0.1% (w/v) DDM (elution buffer).
2	**Purification steps (with corresponding buffer(s) and incubation times)**
A	Harvest total CFS.
B	Incubate with benzonase for 30 min on a wheel, at rt.
C	Centrifuge for 30 min at 20,000 g, 4°C.
D	Harvest the soluble fraction (SN).
E	Equilibrate the Strep-Tactin column (IBA Lifesciences) with 2 CV of **1A** (all steps performed on the bench by gravity).
F	Load SN onto the column.
G	Wash the column with 5 CV of **1A**.
H	Elute the protein of interest with **1B**.

Table 4: Final sample

1	**Yield**
	0.43 mg/mL WGE
1b	**A260/280 ratio**
	1.06
2	**Stability**
	protein has proved unstable during lipid insertion using cyclodextrin for detergent removal
3	**Comment on applicability**
	Solution NMR shows severely broadened resonances hinting to oligomerization or too big protein micelles. Lipid reconstitution is ongoing.



(a) WG-CFPS in presence of detergent and Strep-tag purification of ORF14. SDS-PAGE (upper panel) and WB (lower panel). **(b) SEC profile of ORF14.**

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