

recombinant protein	accession no. full length NCP	calculated MW (# amino acids)	oligonucleotides used for cloning	remarks
SARS-CoV-2 NCPΔ246	QHW06046.1	with tag: 47.3 kDa (413 aa) without tag: 19.5 kDa (175 aa)	5' – AAGTTCTGTTTCAGGGCCCCGACTAAGAAATCTGCTGCTGAGGC – 3' 5' – <u>CTGGTCTAGAAAGCTT</u> TTAGGCCTGAGTTGAGTCAGCAC – 3'	
SARS-CoV-1 NCPΔ247	AAP33707.1	with tag: 47.5 kDa (415 aa) without tag: 19.7 (177 aa)	5' – AAGTTCTGTTTCAGGGCCCCGACTAAGAAATCTGCTGCTGAGGCATC – 3' 5' – <u>CTGGTCTAGAAAGCTT</u> TTATGCCTGAGTTGAATCAGCAGAAG – 3'	
MERS NCPΔ238	AGN70936.1	with tag: 47.8 kDa (415 aa) without tag: 20.0 kDa (177 aa)	5' – AAGTTCTGTTTCAGGGCCCCGACCAAGAAGGACGCCGCGGC – 3' 5' – <u>CTGGTCTAGAAAGCTT</u> TTAGTCCGTATTCACATCAATCATCGG – 3'	codon optimized (<i>E. coli</i>)
HKU1 NCPΔ256	ARU07581.1, S290F	with tag: 48.6 kDa (425 aa) without tag: 20.8 kDa (187 aa)	5' – AAGTTCTGTTTCAGGGCCCCGGCGAAGGAGATCCGCCACAAAATC – 3' 5' – <u>CTGGTCTAGAAAGCTT</u> TTAGGCCACGGAATCTTCCACG – 3'	codon optimized (<i>E. coli</i>)
OC43 NCPΔ258	AMM42450.1, A49T	with tag: 49.2 kDa (430 aa) without tag: 21.4 kDa (192 aa)	5' – AAGTTCTGTTTCAGGGCCCCGGCCAAAGAAGTCAGACAGAAAATTTTG – 3' 5' – <u>CTGGTCTAGAAAGCTT</u> TTATATTTCTGAGGTGTCTTCAGTAAAGG – 3'	
NL63 NCPΔ222	ABI20791.1	with tag: 45.3 kDa (395 aa) without tag: 17.5 kDa (157 aa)	5' – AAGTTCTGTTTCAGGGCCCCGCCGCGCGCGGATAAAC – 3' 5' – <u>CTGGTCTAGAAAGCTT</u> TTAATGCAGCACTTCATTAACGATCTC – 3'	codon optimized (<i>E. coli</i>)
229E NCPΔ235	NP_073556.1	with tag: 45.1 kDa (394 aa) without tag: 17.3 kDa (156 aa)	5' – AAGTTCTGTTTCAGGGCCCCGAGACGAAAGAGCAGAAGCATG – 3' 5' – <u>CTGGTCTAGAAAGCTT</u> TTAATTAACCTTCATCGATGATATCGGTTTC – 3'	codon optimized (<i>E. coli</i>)

Supplemental Table 1. Survey of recombinant CoV NCP proteins. MW: molecular weight; aa: amino acids; *italics*: CoV NCP coding sequence; underlined: 15 bp vector homology sequence for in-fusion cloning.

Serum panel	Germany	Ghana A	Ghana B	Madagascar	Nigeria	Colombia	Lao PDR
Number	139	131	145	166	149	40	20
Sampling year	2004 - 2015	1999	2014 - 2015	2010	2018	2014	2014
Sample type	Serum	Serum	Serum	Plasma	Serum	Serum	Serum
Age (median, IQR)	39 (28-48) [§]	22 (16-44)*	6 (3-7)	24 (20-30)**	42 (30-58) [#]	23 (21-25)	48 (45-53)
Sex m/f (n (%))	72/34 (68/32) [§]	70/61 (53/47)	69/76 (48/52)	166/0 (100/0) [§]	79/70 (53/47)	17/23 (43/57)	3/17 (15/85)
Sampling site	Hamburg	Villages in the central region of Ghana	Agogo	Manakara, Marofarihy, Tsiroanomandidy, Mananjary	Irrua	Valledupar	Savannakhet

Supplemental Table 2. A priori SARS-CoV-2 IgG negative serum/plasma panels. All samples were collected from symptom-free donors prior to the COVID-19 pandemic. IQR: interquartile range (25%-75%); m: male; f: female; n: number.

[§] n=106 (age and sex information not available for 33 donors (all 33: age ≥ 18 years))

* n= 88 (age information not available for 43 donors (all 43: age ≥ 18 years))

** n=164 (age information not available for two donors)

[§] all donors: pregnant women

[#] n=148 (age information not available for one donor)

(A) ELISA 1 (sera 1:100, conjugate 1:20,000)

sample	day 1			day 2			day 3			CV _{intra, av}	mean _{av}	sd of mean _{av}	CV _{inter}
	mean	sd	CV _{intra}	mean	sd	CV _{intra}	mean	sd	CV _{intra}				
<i>pos1a</i>	0.919	0.035	3.8	0.837	0.009	1.0	0.865	0.033	3.8	2.9	0.874	0.042	4.8
<i>pos2a</i>	0.492	0.018	3.6	0.528	0.027	5.2	0.498	0.020	4.1	4.3	0.506	0.020	3.9
<i>pos3a</i>	0.901	0.078	8.6	0.935	0.066	7.1	0.964	0.036	3.7	6.5	0.933	0.032	3.4
<i>pos4a</i>	2.053	0.030	1.5	1.987	0.046	2.3	2.042	0.062	3.0	2.3	2.028	0.035	1.7
<i>neg1a</i>	0.057	0.005	9.1	0.053	0.006	10.8	0.048	0.003	5.3	8.4	0.052	0.005	8.9
<i>neg2a</i>	0.053	0.001	1.9	0.053	0.010	19.2	0.047	0.003	5.6	8.9	0.051	0.004	7.0
<i>neg3a</i>	0.066	0.001	0.9	0.063	0.001	1.8	0.061	0.001	0.9	1.2	0.063	0.002	3.4
<i>neg4a</i>	0.049	0.001	1.2	0.046	0.002	3.8	0.048	0.002	4.4	3.1	0.047	0.001	2.8
										mean_{pos}: 4.0		mean_{pos}: 3.4	
										mean_{neg}: 5.4		mean_{neg}: 5.5	

(B) ELISA 2 (sera 1:2, conjugate 1:50,000)

sample	day 1			day 2			day 3			CV _{intra, av}	mean _{av}	sd of mean _{av}	CV _{inter}
	mean	sd	CV _{intra}	mean	sd	CV _{intra}	mean	sd	CV _{intra}				
<i>pos1b</i>	0.620	0.007	1.2	0.529	0.022	4.1	0.505	0.018	3.6	2.9	0.551	0.061	11.0
<i>pos2b</i>	1.065	0.034	3.2	0.981	0.036	3.7	0.941	0.065	6.9	4.6	0.996	0.064	6.4
<i>pos3b</i>	1.832	0.059	3.2	1.736	0.033	1.9	1.711	0.058	3.4	2.8	1.760	0.064	3.6
<i>pos4b</i>	2.564	0.071	2.8	2.428	0.066	2.7	2.443	0.160	6.5	4.0	2.478	0.075	3.0
<i>neg1b</i>	0.061	0.006	10.0	0.052	0.001	2.2	0.040	0.001	2.5	4.9	0.051	0.011	20.9
<i>neg2b</i>	0.058	0.000	0.0	0.051	0.004	7.8	0.041	0.001	2.8	3.5	0.050	0.008	16.7
<i>neg3b</i>	0.059	0.001	1.0	0.051	0.002	4.1	0.040	0.001	1.4	2.2	0.050	0.009	18.4
<i>neg4b</i>	0.053	0.003	5.8	0.048	0.002	3.2	0.039	0.002	4.0	4.3	0.047	0.007	15.4
										mean_{pos}: 3.6		mean_{pos}: 6.0	
										mean_{neg}: 3.7		mean_{neg}: 17.9	

Supplemental Table 3. Reproducibility of SARS-CoV-2 IgG FcγR ELISAs. A450-A620 values were determined in triplicate for four anti-SARS-CoV-2-NCP IgG positive serum samples and four anti-SARS-CoV-2-NCP IgG negative serum samples on three different days with (A) SARS-CoV-2 IgG FcγR ELISA 1 (sera 1:100, conjugate 1:20,000) and (B) SARS-CoV-2 IgG FcγR ELISA 2 (sera 1:2, conjugate 1:50,000). Sd: standard deviation, av: average; CV_{intra}: intraassay variation, CV_{inter}: interassay variation.