## A SARS-CoV-2 nucleocapsid protein TR-FRET assay amenable to high throughput screening

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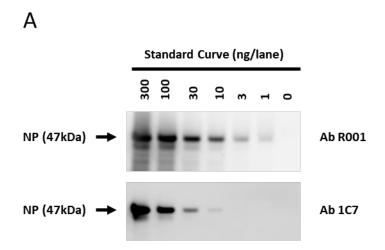
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**Figure S1. Determination of NP concentration in viral samples.** Western blot of recombinant SARS-CoV-2 NP using donor antibody R001 and acceptor antibody 1C7 at 1:1000 dilution.

Α		1h incubation				
		Concentration	5 nM D:	1 nM D:	10 nM D:	2 nM D:
		(ng/mL)	10 nM A	2 nM A	10 nM A	2 nM A
		1500	12.6	43.5	23.5	55.0
		750	8.7	38.7	15.2	54.8
		375	5.2	23.3	8.7	37.1
	Media only	187.5	3.2	12.8	4.9	21.8
	1h	93.8	2.1	7.1	3.1	10.9
		46.9	1.6	4.3	2.0	6.4
		23.4	1.4	2.6	1.6	3.9
		11.7 5.9	1.2	1.9 1.5	1.3	2.5
		2.9	1.0	1.3	1.1	1.7
		1.5	1.0	1.1	1.0	1.2
		0	1.0	1.0	1.0	1.0
			1.0	1.0	1.0	1.0
В				0/N	I 4°C	
_		Concentration	5 nM D:	1 nM D:	10 nM D:	2 nM D:
		(ng/mL)	10 nM A	2 nM A	10 nM A	2 nM A
		1500	9.2	35.1	17.7	45.0
		750	6.6	37.7	11.5	51.1
		375	4.2	26.0	6.4	42.2
	Madia anly	187.5	2.7	12.8	3.8	24.6
	Media only	93.8	1.9	6.5	2.5	11.4
	O/N	46.9	1.5	3.8	1.7	6.1
	0/11	23.4	1.3	2.4	1.4	3.6
		11.7	1.1	1.7	1.2	2.4
		5.9	1.1	1.4	1.0	1.8
		2.9	1.0	1.2	1.0	1.4
		1.5	1.0	1.1	1.0	1.2
		0	1.0	1.0	1.0	1.0
c				1h incı	ubation	
С		Canantation	F mM Di		ubation	2 - 14 D.
С		Concentration	5 nM D:	1 nM D:	10 nM D:	2 nM D:
С		(ng/mL)	10 nM A	1 nM D: 2 nM A	10 nM D: 10 nM A	2 nM A
С		(ng/mL) 1500	10 nM A 15.0	1 nM D: 2 nM A 39.9	10 nM D: 10 nM A 23.8	2 nM A 45.2
С		(ng/mL) 1500 750	10 nM A 15.0 11.3	1 nM D: 2 nM A 39.9 36.5	10 nM D: 10 nM A 23.8 19.0	2 nM A 45.2 50.8
С		(ng/mL) 1500 750 375	10 nM A 15.0 11.3 7.2	1 nM D: 2 nM A 39.9 36.5 29.5	10 nM D: 10 nM A 23.8 19.0 12.4	2 nM A 45.2 50.8 39.6
С	Media + cells	(ng/mL) 1500 750 375 187.5	10 nM A 15.0 11.3 7.2 4.6	1 nM D: 2 nM A 39.9 36.5 29.5 18.5	10 nM D: 10 nM A 23.8 19.0 12.4 7.1	2 nM A 45.2 50.8 39.6 27.6
С		(ng/mL) 1500 750 375	10 nM A 15.0 11.3 7.2	1 nM D: 2 nM A 39.9 36.5 29.5	10 nM D: 10 nM A 23.8 19.0 12.4	2 nM A 45.2 50.8 39.6
С	Media + cells 1h	(ng/mL) 1500 750 375 187.5 93.8	10 nM A 15.0 11.3 7.2 4.6 2.9	1 nM D: 2 nM A 39.9 36.5 29.5 18.5 11.1	10 nM D: 10 nM A 23.8 19.0 12.4 7.1 4.3	2 nM A 45.2 50.8 39.6 27.6 15.2
С		(ng/mL) 1500 750 375 187.5 93.8 46.9	10 nM A 15.0 11.3 7.2 4.6 2.9 2.0	1 nM D: 2 nM A 39.9 36.5 29.5 18.5 11.1 6.0	10 nM D: 10 nM A 23.8 19.0 12.4 7.1 4.3 2.7	2 nM A 45.2 50.8 39.6 27.6 15.2 10.1
С		(ng/mL) 1500 750 375 187.5 93.8 46.9 23.4	10 nM A 15.0 11.3 7.2 4.6 2.9 2.0 1.5	1 nM D: 2 nM A 39.9 36.5 29.5 18.5 11.1 6.0 3.8	10 nM D: 10 nM A 23.8 19.0 12.4 7.1 4.3 2.7	2 nM A 45.2 50.8 39.6 27.6 15.2 10.1 5.1
С		(ng/mL) 1500 750 375 187.5 93.8 46.9 23.4 11.7 5.9	10 nM A 15.0 11.3 7.2 4.6 2.9 2.0 1.5 1.2	1 nM D: 2 nM A 39.9 36.5 29.5 18.5 11.1 6.0 3.8 2.5 1.9	10 nM D: 10 nM A 23.8 19.0 12.4 7.1 4.3 2.7 1.9	2 nM A 45.2 50.8 39.6 27.6 15.2 10.1 5.1 3.8
С		(ng/mL) 1500 750 375 187.5 93.8 46.9 23.4 11.7 5.9 2.9	10 nM A 15.0 11.3 7.2 4.6 2.9 2.0 1.5 1.2 1.1	1 nM D: 2 nM A 39.9 36.5 29.5 18.5 11.1 6.0 3.8 2.5 1.9	10 nM D: 10 nM A 23.8 19.0 12.4 7.1 4.3 2.7 1.9 1.5 1.2 1.1	2 nM A 45.2 50.8 39.6 27.6 15.2 10.1 5.1 3.8 2.5 1.7
C		(ng/mL) 1500 750 375 187.5 93.8 46.9 23.4 11.7 5.9	10 nM A 15.0 11.3 7.2 4.6 2.9 2.0 1.5 1.2 1.1	1 nM D: 2 nM A 39.9 36.5 29.5 18.5 11.1 6.0 3.8 2.5 1.9	10 nM D: 10 nM A 23.8 19.0 12.4 7.1 4.3 2.7 1.9 1.5 1.2	2 nM A 45.2 50.8 39.6 27.6 15.2 10.1 5.1 3.8 2.5 1.7
C		(ng/mL) 1500 750 375 187.5 93.8 46.9 23.4 11.7 5.9 2.9	10 nM A 15.0 11.3 7.2 4.6 2.9 2.0 1.5 1.2 1.1	1 nM D: 2 nM A 39.9 36.5 29.5 18.5 11.1 6.0 3.8 2.5 1.9 1.4	10 nM D: 10 nM A 23.8 19.0 12.4 7.1 4.3 2.7 1.9 1.5 1.2 1.1	2 nM A 45.2 50.8 39.6 27.6 15.2 10.1 5.1 3.8 2.5 1.7
C		(ng/mL) 1500 750 375 187.5 93.8 46.9 23.4 11.7 5.9 2.9 1.5	10 nM A 15.0 11.3 7.2 4.6 2.9 2.0 1.5 1.2 1.1 1.0	1 nM D: 2 nM A 39.9 36.5 29.5 18.5 11.1 6.0 3.8 2.5 1.9 1.4	10 nM D: 10 nM A 23.8 19.0 12.4 7.1 4.3 2.7 1.9 1.5 1.2 1.1	2 nM A 45.2 50.8 39.6 27.6 15.2 10.1 3.8 2.5 1.7 1.4
C		(ng/mL) 1500 750 375 187.5 93.8 46.9 23.4 11.7 5.9 2.9 1.5 0	10 nM A 15.0 11.3 7.2 4.6 2.9 2.0 1.5 1.2 1.1 1.0 1.0	1 nM D: 2 nM A 39.9 36.5 29.5 18.5 11.1 6.0 3.8 2.5 1.9 1.4 1.2 1.0	10 nM D: 10 nM A 23.8 19.0 12.4 7.1 4.3 2.7 1.9 1.5 1.2 1.1 1.0 1.0	2 nM A 45.2 50.8 39.6 27.6 15.2 10.1 5.1 3.8 2.5 1.7 1.4
C		(ng/mL) 1500 750 375 187.5 93.8 46.9 23.4 11.7 5.9 2.9 1.5 0  Concentration (ng/mL)	10 nM A 15.0 11.3 7.2 4.6 2.9 2.0 1.5 1.2 1.1 1.0 1.0	1 nM D: 2 nM A 39.9 36.5 29.5 18.5 11.1 6.0 3.8 2.5 1.9 1.4 1.2 1.0	10 nM D: 10 nM A 23.8 19.0 12.4 7.1 4.3 2.7 1.9 1.5 1.2 1.1 1.0 1.0	2 nM A 45.2 50.8 39.6 27.6 15.2 10.1 5.1 3.8 2.5 1.7 1.4 1.0
C		(ng/mL) 1500 750 375 187.5 93.8 46.9 23.4 11.7 5.9 2.9 1.5 0  Concentration (ng/mL) 1500	10 nM A 15.0 11.3 7.2 4.6 2.9 2.0 1.5 1.2 1.1 1.0 1.0 1.0 1.0	1 nM D: 2 nM A 39.9 36.5 29.5 18.5 11.1 6.0 3.8 2.5 1.9 1.4 1.2 1.0	10 nM D: 10 nM A 23.8 19.0 12.4 7.1 4.3 2.7 1.9 1.5 1.2 1.1 1.0 1.0 1.0 nM D: 10 nM A 22.4	2 nM A 45.2 50.8 39.6 27.6 15.2 10.1 5.1 3.8 2.5 1.7 1.4 1.0
C		(ng/mL) 1500 750 375 187.5 93.8 46.9 23.4 11.7 5.9 2.9 1.5 0  Concentration (ng/mL) 1500 750	10 nM A 15.0 11.3 7.2 4.6 2.9 2.0 1.5 1.2 1.1 1.0 1.0 1.0 1.0 1.0 1.0 nM A 12.0 9.1	1 nM D: 2 nM A 39.9 36.5 29.5 18.5 11.1 6.0 3.8 2.5 1.9 1.4 1.2 1.0 0/N 2 nM A 34.7 42.8	10 nM D: 10 nM A 23.8 19.0 12.4 7.1 4.3 2.7 1.9 1.5 1.2 1.1 1.0 1.0 10 nM D: 10 nM D:	2 nM A 45.2 50.8 39.6 27.6 15.2 10.1 5.1 3.8 2.5 1.7 1.4 1.0 2 nM D: 2 nM A 36.8 48.0
C		(ng/mL) 1500 750 375 187.5 93.8 46.9 23.4 11.7 5.9 2.9 1.5 0  Concentration (ng/mL) 1500 750 375	10 nM A 15.0 11.3 7.2 4.6 2.9 2.0 1.5 1.2 1.1 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0	1 nM D: 2 nM A 39.9 36.5 29.5 18.5 11.1 6.0 3.8 2.5 1.9 1.4 1.2 1.0 0/N 2 nM A 34.7 42.8 37.2	10 nM D: 10 nM A 23.8 19.0 12.4 7.1 4.3 2.7 1.9 1.5 1.2 1.1 1.0 1.0 10 nM D: 10 nM A 22.4 16.6 9.1	2 nM A 45.2 50.8 39.6 27.6 15.2 10.1 5.1 3.8 2.5 1.7 1.4 1.0 2 nM D: 2 nM A 36.8 48.0 49.3
C	1h	(ng/mL) 1500 750 375 187.5 93.8 46.9 23.4 11.7 5.9 2.9 1.5 0  Concentration (ng/mL) 1500 750 375 187.5	10 nM A 15.0 11.3 7.2 4.6 2.9 2.0 1.5 1.2 1.1 1.0 1.0 1.0 1.0 1.0 1.0 5 nM D: 10 nM A 12.0 9.1 5.6 3.7	1 nM D: 2 nM A 39.9 36.5 29.5 18.5 11.1 6.0 3.8 2.5 1.9 1.4 1.2 1.0 0/N 1 nM D: 2 nM A 34.7 42.8 37.2 20.8	10 nM D: 10 nM A 23.8 19.0 12.4 7.1 4.3 2.7 1.9 1.5 1.2 1.1 1.0 1.0 10 nM D: 10 nM A 22.4 16.6 9.1 5.4	2 nM A 45.2 50.8 39.6 27.6 15.2 10.1 5.1 3.8 2.5 1.7 1.4 1.0 2 nM D: 2 nM A 36.8 48.0 49.3 35.1
D	1h  Media + cells	(ng/mL) 1500 750 375 187.5 93.8 46.9 23.4 11.7 5.9 2.9 1.5 0  Concentration (ng/mL) 1500 750 375 187.5 93.8	10 nM A 15.0 11.3 7.2 4.6 2.9 2.0 1.5 1.2 1.1 1.0 1.0 1.0 1.0 1.0 5 nM D: 10 nM A 12.0 9.1 5.6 3.7 2.5	1 nM D: 2 nM A 39.9 36.5 29.5 18.5 11.1 6.0 3.8 2.5 1.9 1.4 1.2 1.0 0/N 1 nM D: 2 nM A 34.7 42.8 37.2 20.8 10.6	10 nM D: 10 nM A 23.8 19.0 12.4 7.1 4.3 2.7 1.9 1.5 1.2 1.1 1.0 1.0 1.0 nM D: 10 nM D: 10 nM A 22.4 16.6 9.1 5.4 3.4	2 nM A 45.2 50.8 39.6 27.6 15.2 10.1 5.1 3.8 2.5 1.7 1.4 1.0 2 nM D: 2 nM A 36.8 48.0 49.3 35.1 17.7
D	1h  Media + cells	(ng/mL) 1500 750 375 187.5 93.8 46.9 23.4 11.7 5.9 2.9 1.5 0  Concentration (ng/mL) 1500 750 375 187.5 93.8 46.9	10 nM A 15.0 11.3 7.2 4.6 2.9 2.0 1.5 1.2 1.1 1.0 1.0 1.0 1.0 1.0 5 nM D: 10 nM A 12.0 9.1 5.6 3.7 2.5 1.7	1 nM D: 2 nM A 39.9 36.5 29.5 18.5 11.1 6.0 3.8 2.5 1.9 1.4 1.2 1.0 0/N 1 nM D: 2 nM A 34.7 42.8 37.2 20.8 10.6 5.7	10 nM D: 10 nM A 23.8 19.0 12.4 7.1 4.3 2.7 1.9 1.5 1.2 1.1 1.0 4°C 10 nM D: 10 nM A 22.4 16.6 9.1 5.4 3.4 2.2	2 nM A 45.2 50.8 39.6 27.6 15.2 10.1 5.1 3.8 2.5 1.7 1.4 1.0 2 nM D: 2 nM A 36.8 48.0 49.3 35.1 17.7 9.5
D	1h	(ng/mL) 1500 750 375 187.5 93.8 46.9 23.4 11.7 5.9 2.9 1.5 0  Concentration (ng/mL) 1500 750 375 187.5 93.8 46.9 23.4	10 nM A 15.0 11.3 7.2 4.6 2.9 2.0 1.5 1.2 1.1 1.0 1.0 1.0 1.0 1.0 5 nM D: 10 nM A 12.0 9.1 5.6 3.7 2.5 1.7	1 nM D: 2 nM A 39.9 36.5 29.5 18.5 11.1 6.0 3.8 2.5 1.9 1.4 1.2 1.0 0/N 1 nM D: 2 nM A 34.7 42.8 37.2 20.8 10.6 5.7 3.3	10 nM D: 10 nM A 23.8 19.0 12.4 7.1 4.3 2.7 1.9 1.5 1.2 1.1 1.0 1.0 10 nM D: 10 nM A 22.4 16.6 9.1 5.4 3.4 2.2 1.6	2 nM A 45.2 50.8 39.6 27.6 15.1 3.8 2.5 1.7 1.4 1.0 2 nM D: 2 nM A 36.8 48.0 49.3 35.1 17.7 9.5 5.3
D	1h  Media + cells	(ng/mL) 1500 750 375 187.5 93.8 46.9 23.4 11.7 5.9 2.9 1.5 0  Concentration (ng/mL) 1500 750 375 187.5 93.8 46.9	10 nM A 15.0 11.3 7.2 4.6 2.9 2.0 1.5 1.2 1.1 1.0 1.0 1.0 1.0 1.0 5 nM D: 10 nM A 12.0 9.1 5.6 3.7 2.5 1.7	1 nM D: 2 nM A 39.9 36.5 29.5 18.5 11.1 6.0 3.8 2.5 1.9 1.4 1.2 1.0 0/N 1 nM D: 2 nM A 34.7 42.8 37.2 20.8 10.6 5.7	10 nM D: 10 nM A 23.8 19.0 12.4 7.1 4.3 2.7 1.9 1.5 1.2 1.1 1.0 4°C 10 nM D: 10 nM A 22.4 16.6 9.1 5.4 3.4 2.2	2 nM A 45.2 50.8 39.6 27.6 15.2 10.1 5.1 3.8 2.5 1.7 1.4 1.0 2 nM D: 2 nM A 36.8 48.0 49.3 35.1 17.7 9.5
D	1h  Media + cells	(ng/mL) 1500 750 375 187.5 93.8 46.9 23.4 11.7 5.9 2.9 1.5 0  Concentration (ng/mL) 1500 750 375 187.5 93.8 46.9 23.4 11.7	10 nM A 15.0 11.3 7.2 4.6 2.9 2.0 1.5 1.2 1.1 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0	1 nM D: 2 nM A 39.9 36.5 29.5 18.5 11.1 6.0 3.8 2.5 1.9 1.4 1.2 1.0 0/N 1 nM D: 2 nM A 34.7 42.8 37.2 20.8 10.6 5.7 3.3 2.3	10 nM D: 10 nM A 23.8 19.0 12.4 7.1 4.3 2.7 1.9 1.5 1.2 1.1 1.0 1.0 4°C 10 nM D: 10 nM A 22.4 16.6 9.1 5.4 3.4 2.2	2 nM A 45.2 50.8 39.6 27.6 15.2 10.1 5.1 3.8 2.5 1.7 1.4 1.0 2 nM D: 2 nM A 36.8 48.0 49.3 35.1 17.7 9.5 5.3 3.3
D	1h  Media + cells	(ng/mL) 1500 750 375 187.5 93.8 46.9 23.4 11.7 5.9 2.9 1.5 0  Concentration (ng/mL) 1500 750 375 187.5 93.8 46.9 23.4 11.7 5.9	10 nM A 15.0 11.3 7.2 4.6 2.9 2.0 1.5 1.2 1.1 1.0 1.0 1.0 1.0 5 nM D: 10 nM A 12.0 9.1 5.6 3.7 2.5 1.7 1.4 1.2 1.0	1 nM D: 2 nM A 39.9 36.5 29.5 18.5 11.1 6.0 3.8 2.5 1.9 1.4 1.2 1.0 O/N 1 nM D: 2 nM A 34.7 42.8 37.2 20.8 10.6 5.7	10 nM D: 10 nM A 23.8 19.0 12.4 7.1 4.3 2.7 1.9 1.5 1.2 1.1 1.0 1.0 4°C 10 nM D: 10 nM A 22.4 16.6 9.1 5.4 2.2 1.6	2 nM A 45.2 50.8 39.6 27.6 15.2 10.1 5.1 3.8 2.5 1.7 1.4 1.0 2 nM D: 2 nM A 36.8 48.0 49.3 35.1 17.7 9.5 5.3 3.3 2.3

**Figure S2. NP HTRF assay is able to detect recombinant SARS-CoV-2 NP. (A)** S/B for Vero E6 NP in media with 1h incubation. **(B)** S/B for Vero E6 NP in media with O/N incubation. **(C)** S/B for Vero E6 NP in media and cells with 1h incubation. **(D)** S/B for Vero E6 NP in media and cells with O/N incubation.

Α	Dilution Factor	Mock TCS	24h TCS	48h TCS
	no dilution	0.8	5.4	9.8
	15	1.1	2.0	8.1
	45	1.0	1.4	3.7
	135	1.1	1.1	2.0
	405	1.0	1.0	1.4
	1215	1.0	1.0	1.1
	3645	1.0	1.0	1.1
	10935	1.0	1.0	1.0
	32805	1.0	1.0	1.0
	98415	1.0	1.0	1.0
	295245	1.0	0.9	1.0
	885735	1.0	1.0	1.0

В				
Ь	Dilution Factor	Mock Lysate	24h Lysate	48h Lysate
	15	0.8	9.7	6.0
	45	1.1	26.8	41.0
	135	1.0	15.8	31.5
	405	1.0	6.0	16.6
	1215	1.0	2.6	6.2
	3645	1.0	1.5	2.5
	10935	1.0	1.2	1.4
	32805	1.0	1.0	1.0
	98415	1.0	1.0	1.0
	295245	1.0	1.0	1.0
	885735	1.0	0.9	0.9
	2657205	1.0	1.0	1.0

**Figure S3.** NP HTRF assay is able to detect SARS-CoV-2 NP in TCS and cell lysates. (A) S/B for Vero E6 TCS. (B) S/B for Vero E6 cell lysate.

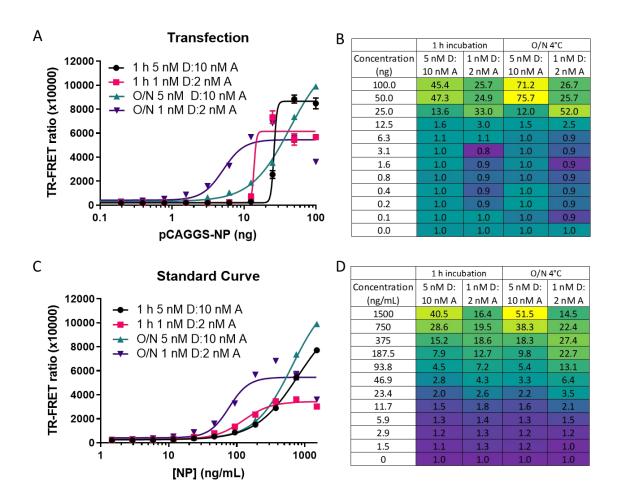


Figure S4. NP HTRF assay is able to detect transiently transfected SARS-CoV-2 NP. (A) TR-FRET ratio from Vero E6 cells transfected with a pCAGGS plasmid encoding SARS-CoV-2 USA-WA1/2020 NP for 24h starting at 100 ng serially diluted 1:2. (B) S/B values for A. (C) TR-FRET ratio from Vero E6 cells treated with SARS-CoV-2 NP starting at 1500 ng/mL and serially diluted 1:2. (D) S/B values for C. Cells were incubated with HTRF reagents for 1h at RT or O/N at 4°C. N=3 wells in a half-area 96-well plate. Error bars indicate S.D.

		P80R	
		$\downarrow$	
USA/WA-1	61	KEDLKFPRGQGVPINTNSS <b>P</b> DDQIGYYRRATRRIRGGDGKMKDLSPRWYF	YYLGTGPEAG 120
Beta	61	KEDLKFPRGQGVPINTNSS <b>P</b> DDQIGYYRRATRRIRGGDGKMKDLSPRWYF	YYLGTGPEAG 120
Gamma	61	${\tt KEDLKFPRGQGVPINTNSS}{\color{red}\underline{\textbf{R}}}{\tt DDQIGYYRRATRRIRGGDGKMKDLSPRWYR}$	YYLGTGPEAG 120
Epsilon	61	KEDLKFPRGQGVPINTNSS <b>P</b> DDQIGYYRRATRRIRGGDGKMKDLSPRWYF	YYLGTGPEAG 120
		R203K G204R T205I	M234I
		$\downarrow$	$\downarrow$
USA/WA-1	181	QASSRSSSRSRNSSRNSTPGSS <b>RGT</b> SPARMAGNGGDAALALLLLDRLNQL	ESK <b>M</b> SGKGQQ 240
Beta	181	QASSRSSSRSRNSSRNSTPGSS <b>RG</b> <u>I</u> SPARMAGNGGDAALALLLLDRLNQLE	ESK <b>M</b> SGKGQQ 240
Gamma	181	QASSRSSRSRNSSRNSTPGSS <u>KR</u> TSPARMAGNGGDAALALLLLDRLNQL	ESK <b>M</b> SGKGQQ 240

181 QASSRSSSRSRNSSRNSTPGSS<mark>KGI</mark>SPARMAGNGGDAALALLLLDRLNQLESK SGKGQQ 240

**Figure S5. Sequence alignment of VoC Beta, Gamma, and Epsilon compared with SARS-CoV-2 USA-WA1/2020**. ClustalO alignment of amino acid sequences for NP regions that were different compared to the reference USA/WA-1 strain. Red, bolded, and underlined letters indicate mutations. Mutation P80R (Gamma), R203K (Gamma, Epsilon), G204R (Gamma), T205I (Beta, Epsilon), and M234I (Epsilon). Sequences were obtained from GISAID.

Epsilon