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

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Supplementary information

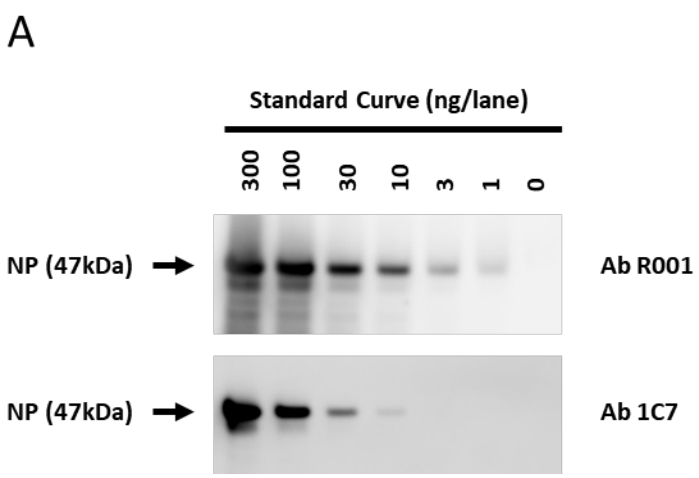
Supplementary Figure 1 - **Determination of NP concentration in viral samples**

Supplementary Figure 2 - **NP HTRF assay is able to detect recombinant SARS-CoV-2 NP**

Supplementary Figure 3 - **NP HTRF assay is able to detect SARS-CoV-2 NP in TCS and cell lysates**

Supplementary Figure 4 - **NP HTRF assay is able to detect transiently transfected SARS-CoV-2 NP**

Supplementary Figure 5 - **Sequence alignment of VoC Beta, Gamma, and Epsilon compared with SARS-CoV-2 USA-WA1/2020**



Supplementary Figure 1. 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.

A

Media only
1h

	1h incubation			
Concentration (ng/mL)	5 nM D: 10 nM A	1 nM D: 2 nM A	10 nM D: 10 nM A	2 nM D: 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
187.5	3.2	12.8	4.9	21.8
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	1.2	1.9	1.3	2.5
5.9	1.1	1.5	1.1	1.7
2.9	1.0	1.3	1.1	1.4
1.5	1.0	1.1	1.0	1.2
0	1.0	1.0	1.0	1.0

B

Media only
O/N

	O/N 4°C			
Concentration (ng/mL)	5 nM D: 10 nM A	1 nM D: 2 nM A	10 nM D: 10 nM A	2 nM D: 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
187.5	2.7	12.8	3.8	24.6
93.8	1.9	6.5	2.5	11.4
46.9	1.5	3.8	1.7	6.1
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

Media + cells
1h

	1h incubation			
Concentration (ng/mL)	5 nM D: 10 nM A	1 nM D: 2 nM A	10 nM D: 10 nM A	2 nM D: 2 nM A
1500	15.0	39.9	23.8	45.2
750	11.3	36.5	19.0	50.8
375	7.2	29.5	12.4	39.6
187.5	4.6	18.5	7.1	27.6
93.8	2.9	11.1	4.3	15.2
46.9	2.0	6.0	2.7	10.1
23.4	1.5	3.8	1.9	5.1
11.7	1.2	2.5	1.5	3.8
5.9	1.1	1.9	1.2	2.5
2.9	1.0	1.4	1.1	1.7
1.5	1.0	1.2	1.0	1.4
0	1.0	1.0	1.0	1.0

D

Media + cells
O/N

	O/N 4°C			
Concentration (ng/mL)	5 nM D: 10 nM A	1 nM D: 2 nM A	10 nM D: 10 nM A	2 nM D: 2 nM A
1500	12.0	34.7	22.4	36.8
750	9.1	42.8	16.6	48.0
375	5.6	37.2	9.1	49.3
187.5	3.7	20.8	5.4	35.1
93.8	2.5	10.6	3.4	17.7
46.9	1.7	5.7	2.2	9.5
23.4	1.4	3.3	1.6	5.3
11.7	1.2	2.3	1.3	3.3
5.9	1.0	1.6	1.1	2.3
2.9	1.0	1.3	1.0	1.6
1.5	0.9	1.2	1.0	1.4
0	1.0	1.0	1.0	1.0

Supplementary Figure 2. 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.

A

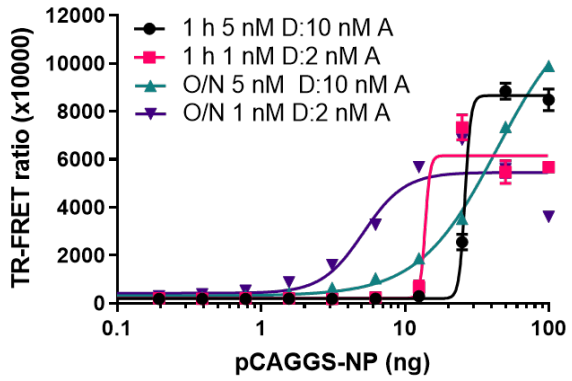
Dilution Factor	Mock TCS	24h TCS	48h TCS
no dilution	0.8	5.4	9.8
15	1.1	2.0	8.1
45	1.0	1.4	3.7
135	1.1	1.1	2.0
405	1.0	1.0	1.4
1215	1.0	1.0	1.1
3645	1.0	1.0	1.1
10935	1.0	1.0	1.0
32805	1.0	1.0	1.0
98415	1.0	1.0	1.0
295245	1.0	0.9	1.0
885735	1.0	1.0	1.0

B

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

Supplementary Figure 3. 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.

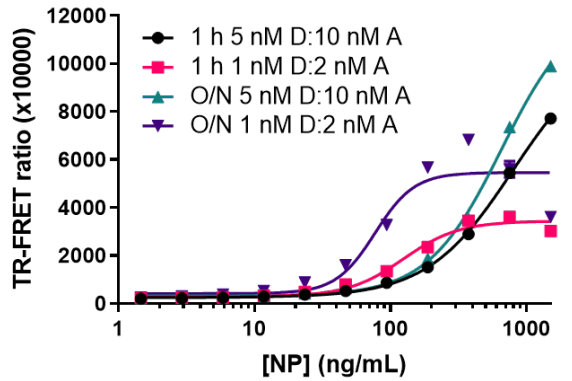
A



B

	1 h incubation		O/N 4°C	
Concentration (ng)	5 nM D: 10 nM A	1 nM D: 2 nM A	5 nM D: 10 nM A	1 nM D: 2 nM A
100.0	45.4	25.7	71.2	26.7
50.0	47.3	24.9	75.7	25.7
25.0	13.6	33.0	12.0	52.0
12.5	1.6	3.0	1.5	2.5
6.3	1.1	1.1	1.0	0.9
3.1	1.0	0.8	1.0	0.9
1.6	1.0	0.9	1.0	0.9
0.8	1.0	0.9	1.0	0.9
0.4	1.0	0.9	1.0	0.9
0.2	1.0	0.9	1.0	0.9
0.1	1.0	1.0	1.0	0.9
0.0	1.0	1.0	1.0	1.0

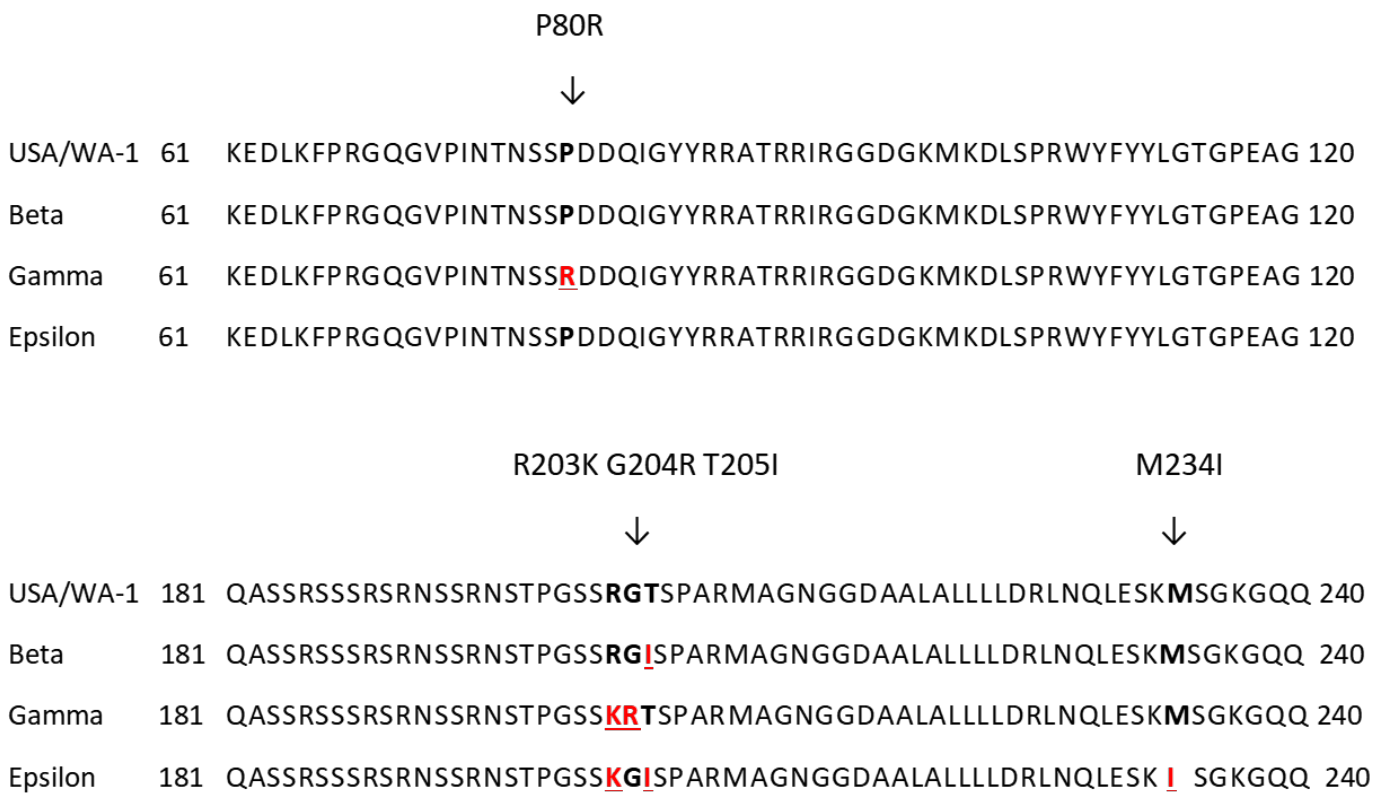
C



D

	1 h incubation		O/N 4°C	
Concentration (ng/mL)	5 nM D: 10 nM A	1 nM D: 2 nM A	5 nM D: 10 nM A	1 nM D: 2 nM A
1500	40.5	16.4	51.5	14.5
750	28.6	19.5	38.3	22.4
375	15.2	18.6	18.3	27.4
187.5	7.9	12.7	9.8	22.7
93.8	4.5	7.2	5.4	13.1
46.9	2.8	4.3	3.3	6.4
23.4	2.0	2.6	2.2	3.5
11.7	1.5	1.8	1.6	2.1
5.9	1.3	1.4	1.3	1.5
2.9	1.2	1.3	1.2	1.2
1.5	1.1	1.3	1.2	1.0
0	1.0	1.0	1.0	1.0

Supplementary Figure 4. 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.



Supplementary Figure 5. 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.