

Supporting Information

Development of a high-throughput homogeneous AlphaLISA drug screening assay for the detection of SARS-CoV-2 Nucleocapsid

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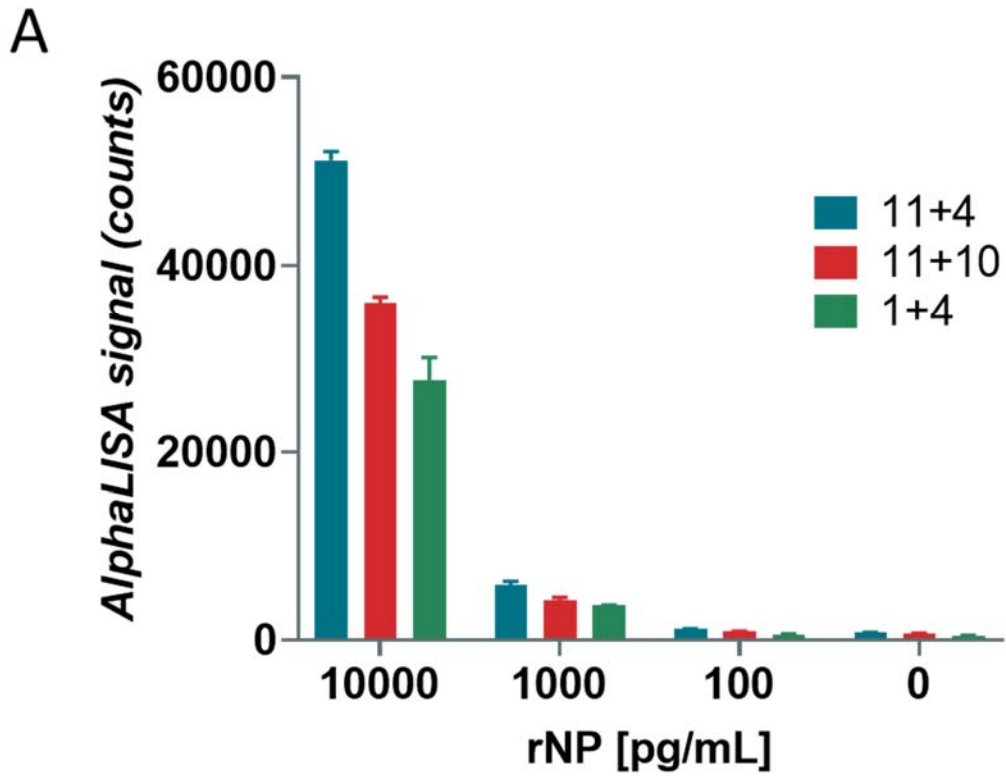
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Supporting Information

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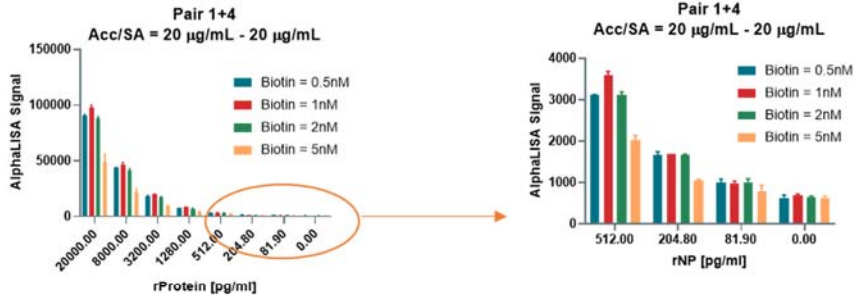


B

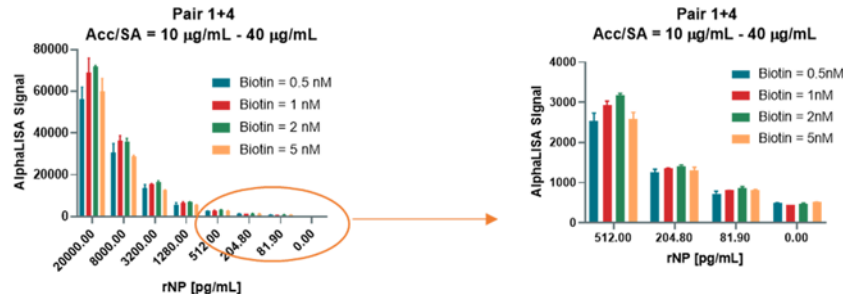
	Signal over Background		
[NP] (pg/mL)	11+4	11+10	1+4
10,000	72.8	51.5	66.2
1,000	8.3	6.0	8.8
100	1.6	1.3	1.5

Figure S-1. Detection of titrated recombinant NP using best antibody pairs. AlphaLISA counts for **(A)** Pair 1+4, Pair 11+4, Pair 11+10 using 10,000, 1000, 100, and 0 pg/mL of NP. **(B)** Signal over Background (S/B) ratios for best antibody pairs in A. N = duplicate wells. Error bars indicate S.D.

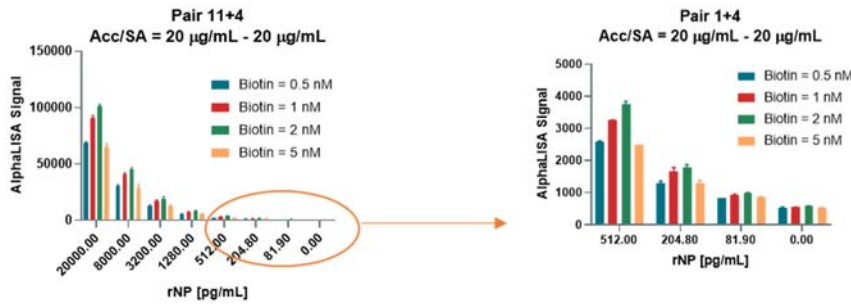
A



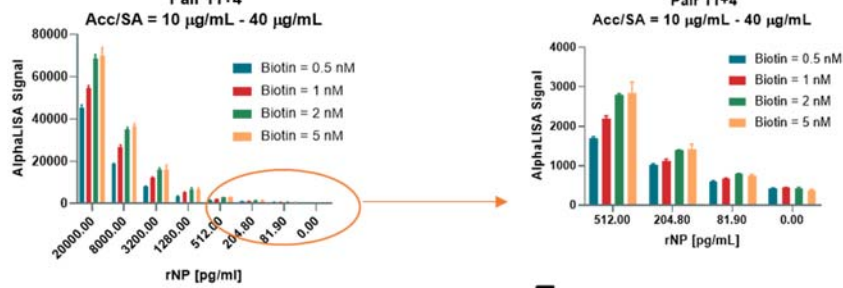
B



C



D



E

Pair 1+4

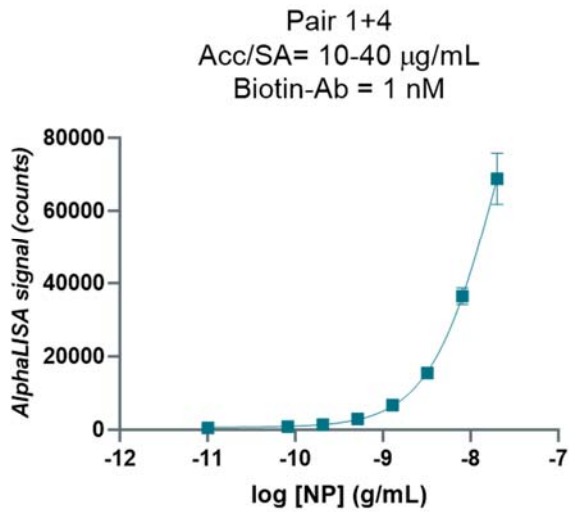
Signal over Background 20 µg/mL - 20 µg/mL			Signal over Background 10 µg/mL - 40 µg/mL		
(pg/mL)	1nM	2nM	(pg/mL)	1nM	2nM
20,000	140.7	136.0	20,000	150.2	148.6
8000.0	67.5	63.8	8000.0	79.6	74.7
3200.0	28.4	26.5	3200.0	33.7	34.4
1280.0	11.7	10.9	1280.0	14.6	14.5
512.0	5.2	4.8	512.0	6.4	6.6
204.8	2.4	2.6	204.8	3.0	2.9
81.9	1.4	1.6	81.9	1.8	1.8
0	1.0	1.0	0	1.0	1.0

F

Pair 11+4

Signal over Background 20 µg/mL - 20 µg/mL			Signal over Background 10 µg/mL - 40 µg/mL		
(pg/mL)	1nM	2nM	(pg/mL)	2nM	5nM
20,000	165.1	174.8	20,000	158.3	182.5
8000.0	74.7	78.5	8000.0	81.0	94.9
3200.0	31.3	33.5	3200.0	37.1	41.2
1280.0	13.8	14.5	1280.0	15.8	17.3
512.0	5.9	6.5	512.0	6.5	7.4
204.8	3.1	3.1	204.8	3.2	3.7
81.9	1.7	1.7	81.9	1.8	2.0
0	1.0	1.0	0	1.0	1.0

Figure S-2. Optimization of best antibody pair and reagent concentrations. AlphaLISA counts for titrated NP using **(A)** 20 µg/mL acceptor and 20 µg/mL or **(B)** 10 µg/mL acceptor and 40 µg/mL Streptavidin for Pair 1+4 and different concentrations of Biotin. Right panel is scaled section of left panel inside of red circle. AlphaLISA counts for titrated NP using **(C)** 20 µg/mL acceptor and 20 µg/mL or **(D)** 10 µg/mL acceptor and 40 µg/mL Streptavidin for Pair 11+4 and different concentrations of Biotin. Right panel is scaled section of left panel inside of red circle. **(E)** S/B ratios for A and B using 1 nM and 2 nM Biotin. **(F)** S/B ratios for A and B using 1 nM and 2 nM Biotin. N = duplicate wells. Error bars indicate S.D.

A**C**

Signal over Background		
(pg/mL)	1+4	11+4
20,000	150.2	182.5
8000.0	79.6	94.9
3200.0	33.7	41.2
1280.0	14.6	17.3
512.0	6.4	7.4
204.8	3.0	3.7
81.9	1.8	2.0
0	1.0	1.0

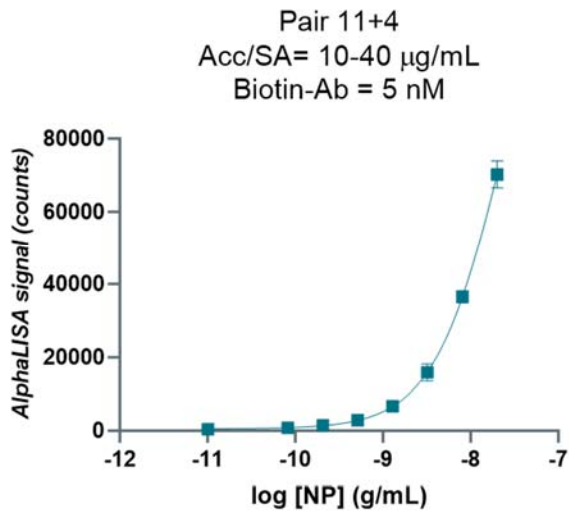
B

Figure S-3. Standard curves using best conditions for best antibody pairs. **(A)** AlphaLISA signal (counts) for titrated NP (g/mL) from 20,000 pg/mL to 0 pg/mL using ACC/SA = 10-40 µg/mL and 1 nM Biotin-Ab for Pair 1+4. **(B)** AlphaLISA signal (counts) for titrated NP (g/mL) from 20,000 pg/mL to 0 pg/mL using ACC/SA = 10-40 µg/mL and 5 nM Biotin for Pair 11+4. **(C)** S/B ratios for graphs 1+4 and 11+4. N = duplicate wells. Error bars indicate S.D.

A

Signal over Background		
(ng/mL)	AlphaLISA LB	NCATSLB
200000.0	4.1	7.4
40000.0	20.3	29.0
8000.0	65.0	105.2
1600.0	152.1	296.8
320.0	233.3	514.7
64.0	167.4	355.7
12.8	88.9	74.3
2.6	36.5	8.9
0.5	12.0	2.4
0.1	6.4	1.3
0.02	3.0	1.1

B

Interpolated Concentration (ng/mL)	
24 hr TCS	112.6
48 hr TCS	981.7
24 hr Lysate	4,575
48 hr Lysate	35,276

C

Signal over Background		
(ng/mL)	AlphaLISA LB	NCATSLB
200000.0	28.2	28.1
40000.0	69.6	88.1
8000.0	221.9	251.6
1600.0	462.1	584.1
320.0	381.3	644.7
64.0	319.2	376.2
12.8	141.5	87.4
2.6	39.9	10.1
0.5	8.6	3.1
0.1	3.0	1.3
0.02	2.4	1.0

D

Interpolated Concentration (ng/mL)	
24 hr TCS	80.3
48 hr TCS	707.6
24 hr Lysate	7,382.7
48 hr Lysate	30,155.8

Table S-1 SARS-CoV-2 Nucleocapsid detection in virally infected cell lysates and tissue culture supernatants. **(A)** S/B ratios for Figure 1A. Values in red show the concentrations at which accurate determination of NP is not possible due to the hook effect. **(B)** Interpolated concentrations in ng/mL of NP in TCS and Lysates collected at 24 or 48 hours using the curve from A. **(C)** S/B ratios for Figure 1C. Values in red show the concentrations at which accurate determination of NP is not possible due to the hook effect. **(D)** Interpolated concentrations in ng/mL of NP in TCS and Lysates collected at 24 or 48 hours using the curve from B.

A

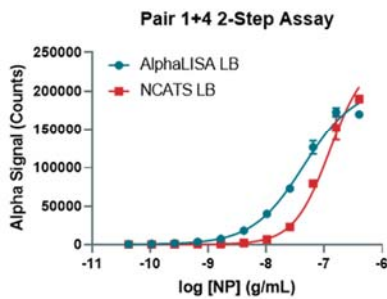
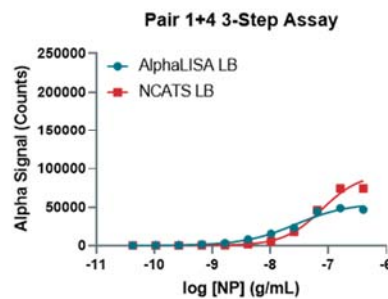
Dispensing protocol (2-step assay):

1. Dispense 5 μ l of protein
2. Dispense 10 μ l of mix of acceptor beads and biotinylated antibody
Incubate 60 min at RT
3. Dispense 5 μ l of SA-Donor Beads
Incubate 30 min at RT
4. Read plates on EnVision

B

Dispensing protocol (3-step assay):

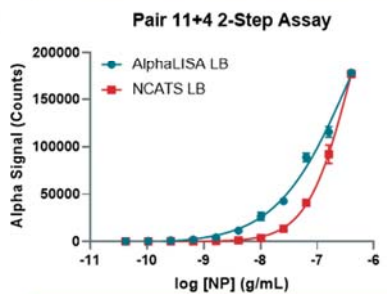
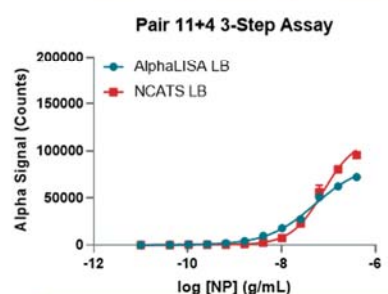
1. Dispense 5 μ l of protein
2. Dispense 5 μ l of acceptor beads
Incubate 60 min at RT
3. Dispense 5 μ l of biotinylated antibody
Incubate 60 min at RT
4. Dispense 5 μ l of SA-Donor Beads
Incubate 30 min at RT
5. Read plates on EnVision

C**E****D**

Signal over Background		
(ng/mL)	AlphaLISA LB	NCATS LB
400.0	233.1	366.0
160.00	241.8	366.0
64.00	222.4	228.5
25.60	115.8	89.8
10.24	77.1	28.4
4.10	40.6	10.0
1.64	17.2	4.3
0.66	8.6	2.3
0.26	4.1	1.5
0.10	2.2	1.2
0.04	1.5	1.1
0.0	1.0	1.0

F

Signal over Background		
(ng/mL)	AlphaLISA LB	NCATS LB
400.0	432.7	537.5
160.00	439.6	429.9
64.00	321.7	224.1
25.60	185.4	65.1
10.24	101.8	19.1
4.10	46.6	6.4
1.64	19.4	2.9
0.66	9.3	1.8
0.26	4.3	1.3
0.10	2.3	1.1
0.04	1.4	1.0
0.0	1.0	1.0

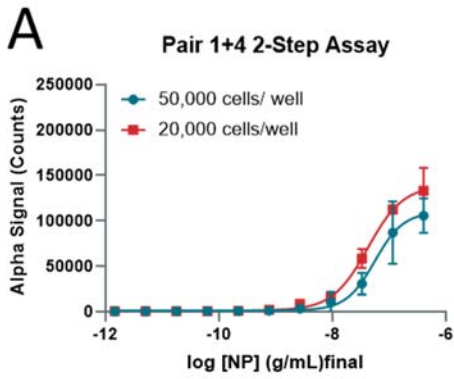
G**I****H**

Signal over Background		
(ng/mL)	AlphaLISA LB	NCATS LB
400.0	630.3	626.3
160.00	409.2	325.9
64.00	314.2	145.2
25.60	151.7	48.0
10.24	94.4	13.4
4.10	40.9	4.9
1.64	15.6	2.6
0.66	7.4	1.7
0.26	3.6	1.3
0.10	2.0	1.1
0.04	1.7	1.1
0.0	1.0	1.0

J

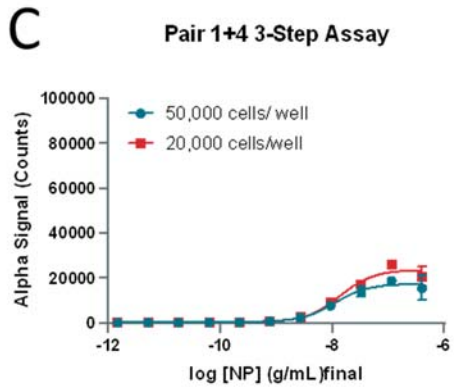
Signal over Background		
(ng/mL)	AlphaLISA LB	NCATS LB
400.0	263.6	348.5
160.00	227.7	293.7
64.00	186.4	204.9
25.60	100.5	84.2
10.24	65.8	27.9
4.10	36.2	10.1
1.64	16.2	4.3
0.66	7.7	2.1
0.26	3.8	1.4
0.10	2.6	1.5
0.04	1.7	1.1
0.0	1.0	0.8

Figure S-4 2-step vs 3-step assay optimization for best antibody pairs and conditions. Dispensing protocols for **(A)** 2-step and **(B)** 3-step assay. **(C)** Standard curve for NP using Pair 1+4 and two-step assay protocol using AlphaLISA or NCATS LBs. **(D)** S/B ratios for C. **(E)** Standard curve for NP using Pair 1+4 and three-step assay protocol using AlphaLISA or NCATS LBs. **(F)** S/B ratios for E. **(G)** Standard curve for NP using Pair 11+4 and two-step assay protocol using AlphaLISA or NCATS LBs. **(H)** S/B ratios for G. **(I)** Standard curve for NP using Pair 11+4 and three-step assay protocol using AlphaLISA or NCATS LBs. **(J)** S/B ratios for I. N = triplicate wells. Error bars indicate S.D.



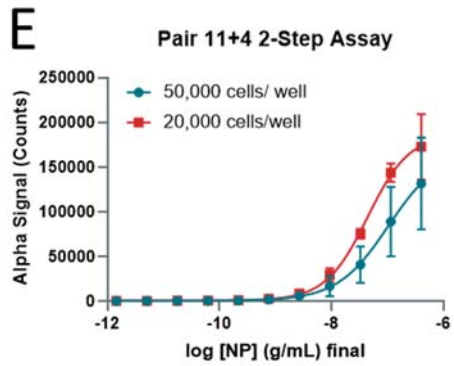
B

Signal over Background		
Final conc.(ng/mL)	50,000 cells/well	20,000 cells/well
400	253.8	283.5
114.286	209.2	239.9
32.653	74.1	124.4
9.329	24.7	33.2
2.666	8.1	17.9
0.762	2.6	4.7
0.218	1.5	1.8
0.062	1.9	1.2
0.018	1.5	1.0
0.005	1.0	1.0
0.001	1.0	1.0
0	1.0	1.0



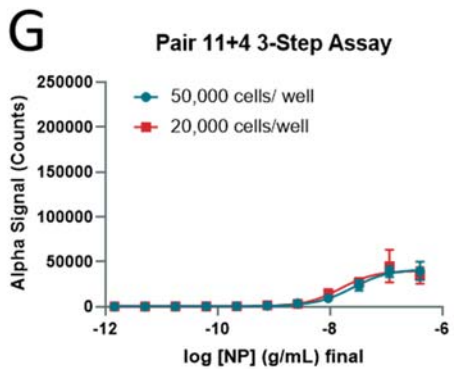
D

Signal over Background		
Final conc.(ng/mL)	50,000 cells/well	20,000 cells/well
400	68.3	88.9
114.286	83.2	112.7
32.653	62.0	73.9
9.329	34.3	39.0
2.666	11.0	11.4
0.762	3.8	4.3
0.218	1.6	1.7
0.062	1.2	1.2
0.018	1.0	1.0
0.005	0.9	0.9
0.001	1.0	1.0
0	1.0	1.0



F

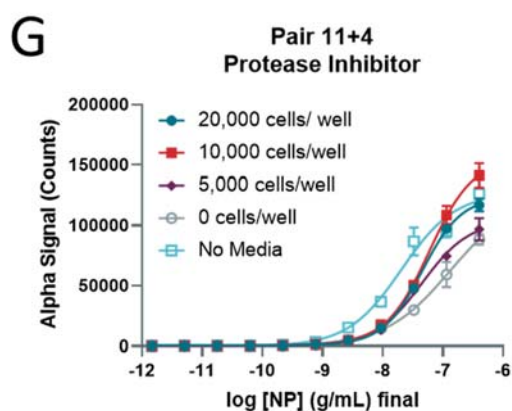
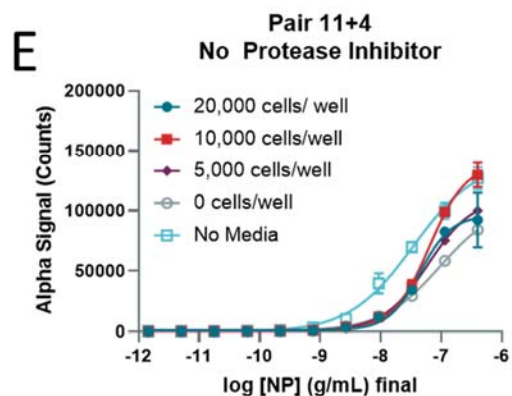
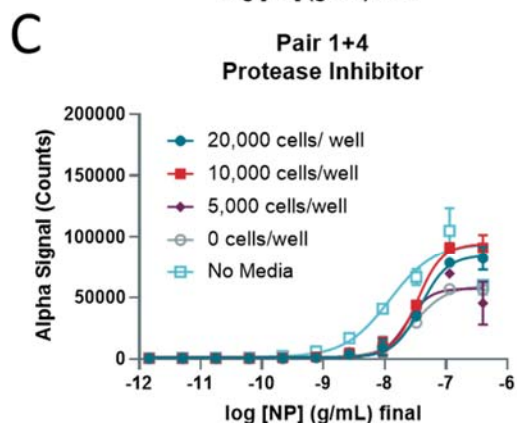
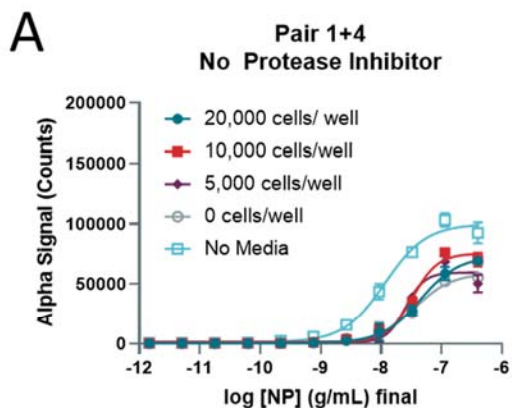
Signal over Background		
Final conc.(ng/mL)	50,000 cells/well	20,000 cells/well
400	303.4	447.5
114.286	205.2	372.5
32.653	94.3	195.1
9.329	39.3	76.2
2.666	14.3	22.9
0.762	4.6	7.2
0.218	1.6	2.8
0.062	1.2	1.7
0.018	1.0	1.2
0.005	0.8	1.0
0.001	0.9	1.1
0	1.0	1.0



H

Signal over Background		
Final conc.(ng/mL)	50,000 cells/well	20,000 cells/well
400	195.5	153.7
114.286	192.0	199.6
32.653	116.4	118.6
9.329	46.5	65.7
2.666	17.8	14.1
0.762	5.3	6.0
0.218	2.1	1.9
0.062	1.2	1.3
0.018	1.0	1.0
0.005	1.0	0.9
0.001	1.0	0.9
0	1.0	1.0

Figure S-5 Simulation of viral infection in 384 well plates using recombinant NP spiked into Vero-E6 wells. **(A)** Detection of titrated NP in wells culturing 50,000 or 20,000 Vero-E6 cells using the two-step assay for Pair 1+4. **(B)** S/B ratios from A. **(C)** Detection of titrated NP in wells culturing 50,000 or 20,000 Vero-E6 cells using the three-step assay for Pair 1+4. **(D)** S/B ratios from C. **(E)** Detection of titrated NP in wells culturing 50,000 or 20,000 Vero-E6 cells using the two-step assay for Pair 11+4. **(F)** S/B ratios from E. **(G)** Detection of titrated NP in wells culturing 50,000 or 20,000 Vero-E6 cells using the three-step assay for Pair 11+4. **(H)** S/B ratios from G. N = triplicate wells. Error bars indicate S.D.



B

Signal over Background					
(ng/mL) final	20,000 cells	10,000 cells	5,000 cells	Media	No Media
400.00	172.7	179.2	125.1	131.5	119.6
114.29	146.9	194.4	170.1	123.8	133.5
32.65	70.2	90.0	97.0	61.2	98.9
9.33	24.7	29.9	17.3	24.3	56.5
2.67	6.4	9.7	9.7	9.9	20.2
0.76	2.2	2.6	3.1	2.5	7.8
0.22	1.2	1.3	1.5	1.4	3.1
0.06	0.9	1.1	1.1	1.1	1.5
0.02	0.9	1.0	0.9	1.0	1.1
0.01	0.9	1.0	0.9	0.9	1.1
0.001	0.9	0.9	0.9	1.0	1.0
0	1.0	1.0	1.0	1.0	1.0

D

Signal over Background					
(ng/mL) final	20,000 cells	10,000 cells	5,000 cells	Media	No Media
400.00	217.3	240.8	134.5	142.5	68.5
114.29	207.6	240.6	205.9	143.1	161.3
32.65	91.6	115.8	112.3	74.5	102.6
9.33	24.0	26.8	29.2	24.8	62.8
2.67	10.0	12.7	11.7	12.5	26.1
0.76	3.1	4.0	3.9	3.4	9.4
0.22	1.5	1.7	1.9	1.6	3.4
0.06	1.1	1.2	1.3	1.2	1.8
0.02	1.0	1.1	1.2	1.2	1.3
0.01	0.9	1.1	1.2	1.1	1.1
0.001	1.0	1.0	1.2	1.0	1.0
0	1.0	1.0	1.0	1.0	1.0

F

Signal over Background					
(ng/mL) final	20,000 cells	10,000 cells	5,000 cells	Media	No Media
400.00	272.6	377.3	271.8	254.8	385.2
114.29	243.9	287.2	204.8	176.8	303.1
32.65	101.2	108.8	99.6	89.0	211.9
9.33	35.1	34.9	30.6	37.8	120.9
2.67	9.7	10.8	11.1	13.6	31.7
0.76	2.7	3.2	3.2	3.2	10.6
0.22	1.4	1.6	1.6	1.6	3.4
0.06	1.0	1.1	1.1	1.2	1.6
0.02	0.9	1.0	0.9	1.1	1.1
0.01	0.9	1.0	0.9	1.1	1.1
0.001	0.9	1.0	0.8	1.0	1.1
0	1.0	1.0	1.0	1.0	1.0

H

Signal over Background					
(ng/mL) final	20,000 cells	10,000 cells	5,000 cells	Media	No Media
400.00	346.4	428.5	284.4	266.8	385.3
114.29	288.4	329.5	219.3	178.7	290.5
32.65	142.8	153.8	135.3	90.0	264.4
9.33	45.3	54.4	39.4	44.1	111.7
2.67	13.3	15.0	14.2	14.8	46.5
0.76	4.5	4.9	4.3	4.4	10.5
0.22	1.9	2.3	1.9	1.7	3.2
0.06	1.2	1.4	1.3	1.2	1.5
0.02	1.0	1.0	1.0	1.0	1.2
0.01	1.0	1.1	1.0	1.0	1.1
0.001	1.0	1.0	0.9	1.0	1.0
0	1.0	1.0	1.0	1.0	1.0

Figure S-6 Optimization of LB and cell density for 384-well plate viral infection simulation using recombinant NP. **(A)** Detection of titrated NP in wells culturing 20,000, 10,000, 5000, or 0 cells/well along with a no-media control without protease inhibitor in Vero-E6 cells using Pair 1+4. **(B)** S/B ratios from A. Red values indicate hook effect or below the lower limit of detection for the higher and lower concentrations of NP, respectively. **(C)** Detection of titrated NP in wells culturing 20,000, 10,000, 5000, or 0 cells/well along with a no-media control with protease inhibitor in Vero-E6 cells using Pair 1+4. **(D)** S/B ratios from C. Red values indicate hook effect or below the lower limit of detection for the higher and lower concentrations of NP, respectively. **(E)** Detection of titrated NP in wells culturing 20,000, 10,000, 5000, or 0 cells/well along with a no-media control without protease inhibitor in Vero-E6 cells using Pair 11+4. **(F)** S/B ratios from E. Red values indicate signals below the lower limit of detection for lower concentrations of NP. **(G)** Detection of titrated NP in wells culturing 20,000, 10,000, 5000, or 0 cells/well along with a no-media control with protease inhibitor in Vero-E6 cells using Pair 11+4. **(H)** S/B ratios from G. Red values indicate signals below the lower limit of detection for lower concentrations of NP. N = triplicate wells. Error bars indicate S.D.

A

Signal over Background			
(ng/mL) final	20 uL final	50 uL final	100 uL final
500.00	309.8	121.0	165.7
142.86	453.5	134.1	218.3
40.82	456.9	114.1	176.5
11.66	332.7	63.7	130.7
3.33	149.7	32.4	82.6
0.95	52.1	14.8	36.3
0.27	15.7	5.4	13.3
0.08	5.2	2.5	4.9
0.02	2.3	2.0	2.4
0.01	1.3	1.2	1.5
0.002	1.1	1.1	1.2
0.00	1.0	1.0	1.0

B

Signal over Background			
(ng/mL) final	20 uL	50 uL	100 uL
500.00	578.7	210.3	386.6
142.86	639.7	145.9	322.6
40.82	571.5	91.5	205.9
11.66	349.1	62.0	127.9
3.33	160.9	36.2	86.6
0.95	55.2	16.1	37.5
0.27	17.1	6.1	14.7
0.08	5.9	2.7	5.2
0.02	2.4	1.5	2.8
0.01	1.4	1.2	1.6
0.002	1.0	1.1	1.2
0.00	1.0	1.0	1.0

Table S-2 Optimization of assay volumes for 384-well plate using recombinant Nucleocapsid. **(A)** S/B ratios from Figure 3A. Red values indicate hook effect (upper limit) and lower limit of detection. **(B)** S/B ratios from Figure 3C. Red values indicate lower limit of detection. N = triplicate wells. Error bars indicate S.D.

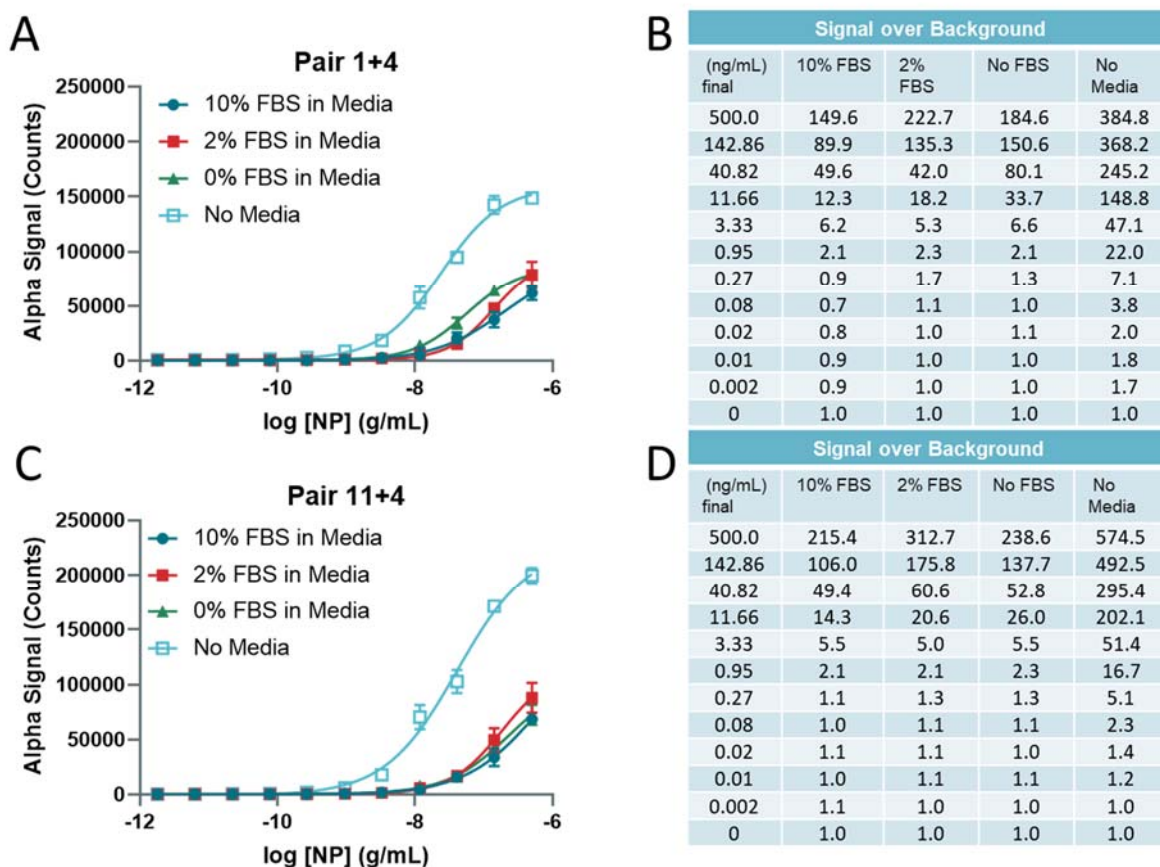


Figure S-7 Optimization of AlphaLISA assay using different FBS percentages in cell culture media. **(A)** Detection of titrated NP in wells with 10%, 2% and 0% FBS in media as well as no media control using Pair 1+4. Assay conducted using the AlphaLISA LB. **(B)** S/B ratios from A. **(C)** Detection of titrated NP in wells with 10%, 2% and 0% FBS in media as well as no media control using Pair 1+4. Assay conducted using the AlphaLISA LB. **(D)** S/B ratios from A. N = duplicate wells. Error bars indicate S.D.

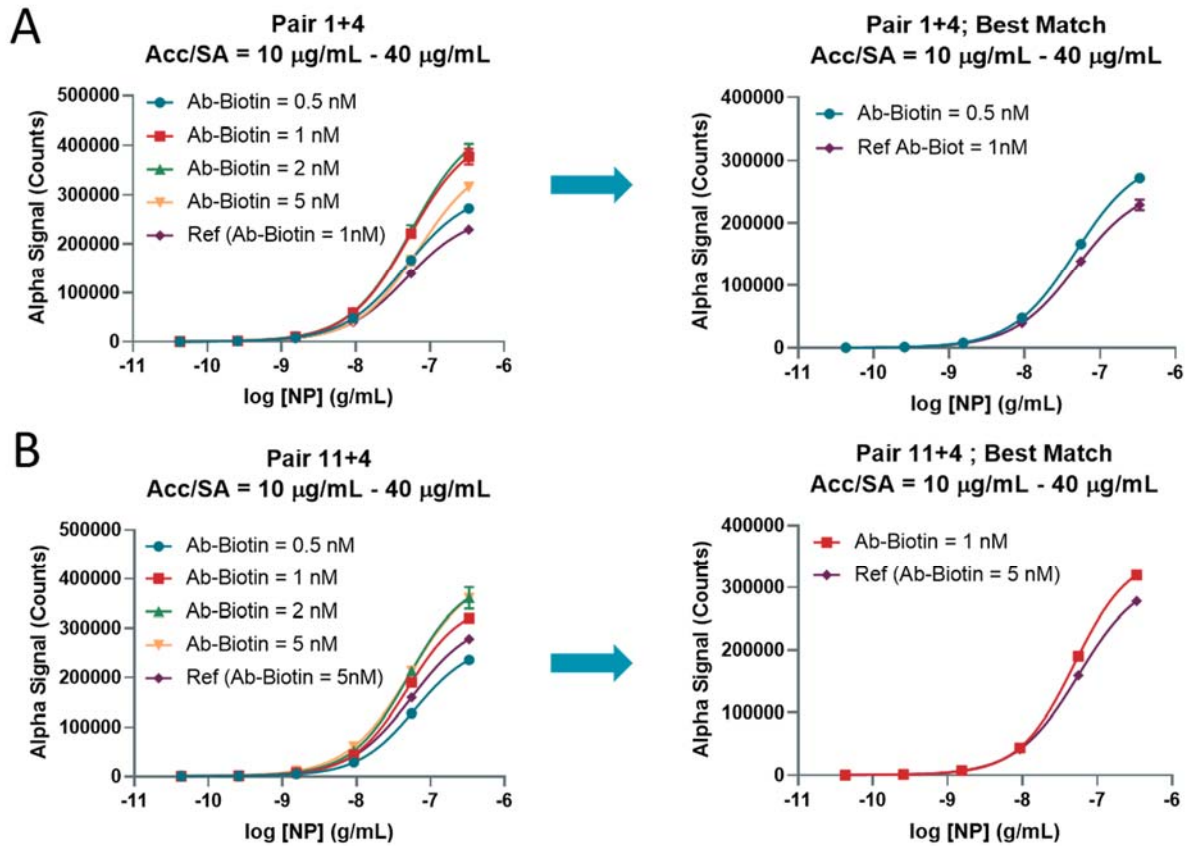


Figure S-8 Optimization of best antibody pair and reagent concentrations of second preparation to match first preparation. **(A)** AlphaLISA counts for titrated NP using 10 µg/mL acceptor and 40 µg/mL Streptavidin for Pair 1+4 and different concentrations of Biotin. Right panel comparing the best condition from first batch to matching condition from second batch. **(B)** AlphaLISA counts for titrated NP using 10 µg/mL acceptor and 40 µg/mL Streptavidin for Pair 11+4 and different concentrations of Biotin. Right panel comparing the best condition from first batch to matching condition from second batch. N = duplicate wells. Error bars indicate S.D.

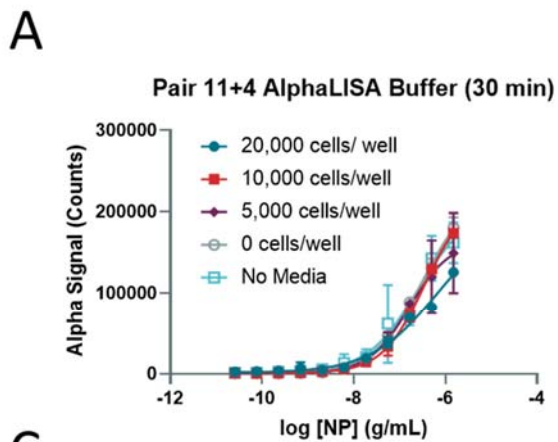
A

Signal over Background					
(ng/mL) final	20,000 cells	10,000 cells	5,000 cells	Media	No Media
500.0	205.3	284.7	255.2	300.8	94.8
142.86	141.3	208.0	176.0	193.2	101.9
40.82	88.4	124.7	126.4	103.4	110.0
11.66	38.4	50.8	45.3	37.2	79.3
3.33	13.3	17.9	14.7	14.2	37.5
0.95	5.8	5.8	5.4	5.0	13.2
0.27	2.9	2.8	2.2	2.3	5.0
0.08	4.0	1.6	1.4	1.7	2.2
0.02	2.6	1.7	1.1	1.5	1.5
0.01	1.6	1.9	1.0	1.2	1.1
0.00	1.0	1.6	1.0	1.3	1.1
0.0	1.0	1.0	1.0	1.0	1.0

B

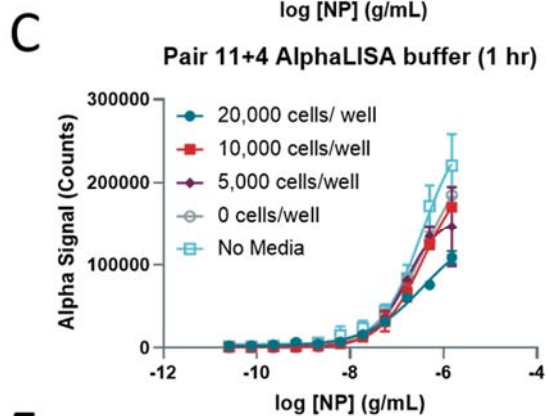
Signal over Background					
(ng/mL) final	20,000 cells	10,000 cells	5,000 cells	Media	No Media
500.0	331.6	325.4	309.5	245.2	205.1
142.86	228.6	233.1	207.1	169.7	182.4
40.82	119.7	102.4	74.3	79.3	128.6
11.66	50.6	39.3	36.9	25.0	70.3
3.33	16.0	14.2	13.4	10.6	28.3
0.95	6.0	4.4	5.1	4.4	9.9
0.27	2.7	2.2	2.1	2.5	4.0
0.08	2.0	1.3	1.4	2.4	1.8
0.02	2.4	1.6	1.1	2.7	1.2
0.01	1.2	1.2	1.1	3.1	0.9
0.00	1.3	1.0	1.1	2.2	0.8
0.0	1.0	1.0	1.0	1.0	1.0

Table S-3 Optimization of 384-well plate cell number in simulated infection using second preparation of reagents. **(A)** S/B ratios from Figure 4A. **(B)** S/B ratios from Figure 4B. N = triplicate wells. Error bars indicate S.D.



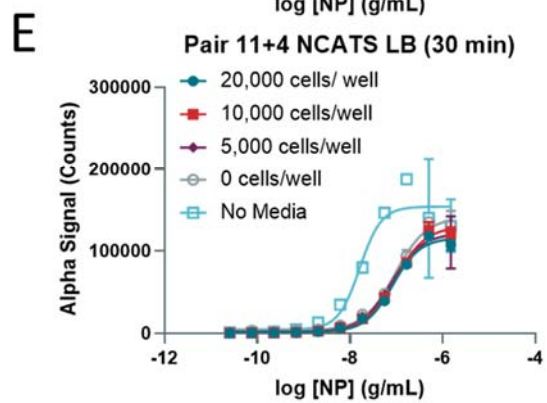
B

Signal over Background					
(ng/mL) final	20,000 cells	10,000 cells	5,000 cells	Media	No Media
1500.0	35.8	77.0	137.2	290.4	183.8
500.00	24.9	56.1	102.9	202.6	143.2
166.67	19.8	30.3	75.9	130.4	69.7
55.56	10.4	14.3	34.5	64.1	60.3
18.52	5.3	5.9	14.2	22.6	19.8
6.17	2.4	2.4	5.6	8.7	12.6
2.06	1.6	1.0	2.1	2.7	5.2
0.69	2.2	0.6	1.2	1.8	2.9
0.23	1.2	0.4	0.9	1.2	2.1
0.08	0.9	0.4	0.8	1.0	1.2
0.03	0.8	0.5	0.8	0.9	1.3
0.0	1.0	1.0	1.0	1.0	1.0



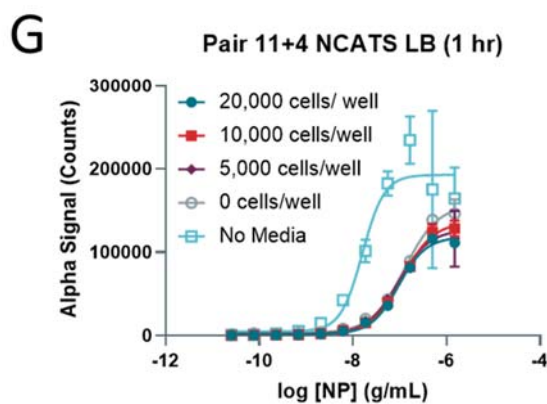
D

Signal over Background					
(ng/mL) final	20,000 cells	10,000 cells	5,000 cells	Media	No Media
1500.0	43.2	87.5	204.4	326.5	146.0
500.00	28.2	65.2	163.9	237.8	128.9
166.67	23.9	36.2	118.7	160.6	64.3
55.56	13.1	17.0	56.2	84.6	55.6
18.52	6.6	7.2	23.4	30.3	18.2
6.17	2.7	2.9	9.2	11.7	11.9
2.06	1.5	1.2	3.3	3.2	4.9
0.69	2.4	0.6	1.5	2.0	2.7
0.23	1.3	0.4	1.0	1.3	2.2
0.08	1.0	0.4	0.9	1.0	1.3
0.03	0.8	0.5	0.9	1.0	1.3
0.0	1.0	1.0	1.0	1.0	1.0



F

Signal over Background					
(ng/mL) final	20,000 cells	10,000 cells	5,000 cells	Media	No Media
1500.0	212.0	262.9	233.0	298.3	118.1
500.00	234.1	271.4	263.4	306.1	126.6
166.67	165.8	185.6	179.5	212.9	169.1
55.56	78.4	94.0	98.3	109.0	132.3
18.52	35.5	38.7	42.9	52.0	72.3
6.17	12.6	15.2	17.1	21.9	31.5
2.06	4.6	5.9	6.4	11.3	12.0
0.69	2.1	2.5	3.3	7.9	4.5
0.23	1.3	1.5	1.8	3.0	2.0
0.08	1.1	1.1	1.2	2.1	1.1
0.03	0.9	1.0	1.0	1.5	0.9
0.0	1.0	1.0	1.0	1.0	1.0

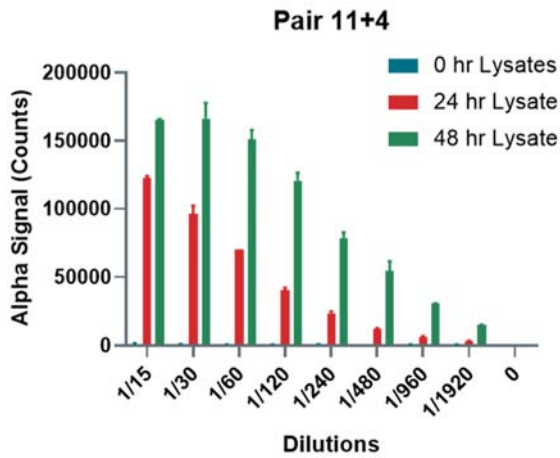


H

Signal over Background					
(ng/mL) final	20,000 cells	10,000 cells	5,000 cells	Media	No Media
1500.0	187.1	259.2	225.2	267.2	137.2
500.00	195.3	251.6	245.8	254.0	146.1
166.67	137.1	167.3	157.8	163.3	195.5
55.56	60.8	80.4	84.5	79.6	152.3
18.52	26.6	31.0	34.3	36.7	84.8
6.17	9.2	12.1	13.8	15.3	35.6
2.06	3.6	5.0	5.2	7.8	12.5
0.69	1.9	2.5	2.9	5.4	4.7
0.23	1.2	1.4	1.7	2.2	1.9
0.08	1.1	1.1	1.2	1.6	1.0
0.03	0.9	1.0	1.1	1.2	0.7
0.0	1.0	1.0	1.0	1.0	1.0

Figure S-9 Optimization of LB, cell density, and incubation time for 384-well plate viral infection simulation using recombinant NP and second batch of reagents. **(A)** Detection of titrated NP in wells culturing 20,000, 10,000, 5000, or 0 cells/well along with a no-media control in Vero-E6 cells using Pair 11+4 and AlphaLISA LB after 30 min. **(B)** S/B ratios from A. **(C)** Detection of titrated NP in wells culturing 20,000, 10,000, 5000, or 0 cells/well along with a no-media control in Vero-E6 cells using Pair 11+4 and AlphaLISA buffer after 1 hr. **(D)** S/B ratios from C. **(E)** Detection of titrated NP in wells culturing 20,000, 10,000, 5000, or 0 cells/well along with a no-media control in Vero-E6 cells using Pair 11+4 and NCATS LB after 30 min. **(F)** S/B ratios from E. **(G)** Detection of titrated NP in wells culturing 20,000, 10,000, 5000, or 0 cells/well along with a no media control in Vero-E6 cells using Pair 11+4 after 1 hr. **(H)** S/B ratios from G. N = triplicate wells. Error bars indicate S.D.

A



B

Signal over Background			
Sample dilution	0 hr Lysates	24 hr Lysates	48 hr Lysates
1/15	2.4	276.8	371.2
1/30	2.1	216.5	373.2
1/60	1.8	158.0	339.3
1/120	1.8	91.3	271.4
1/240	1.8	53.0	176.6
1/480	1.4	27.6	122.9
1/960	1.3	14.8	68.8
1/1920	1.2	7.6	33.7
0.0	1.0	1.0	1.0

C

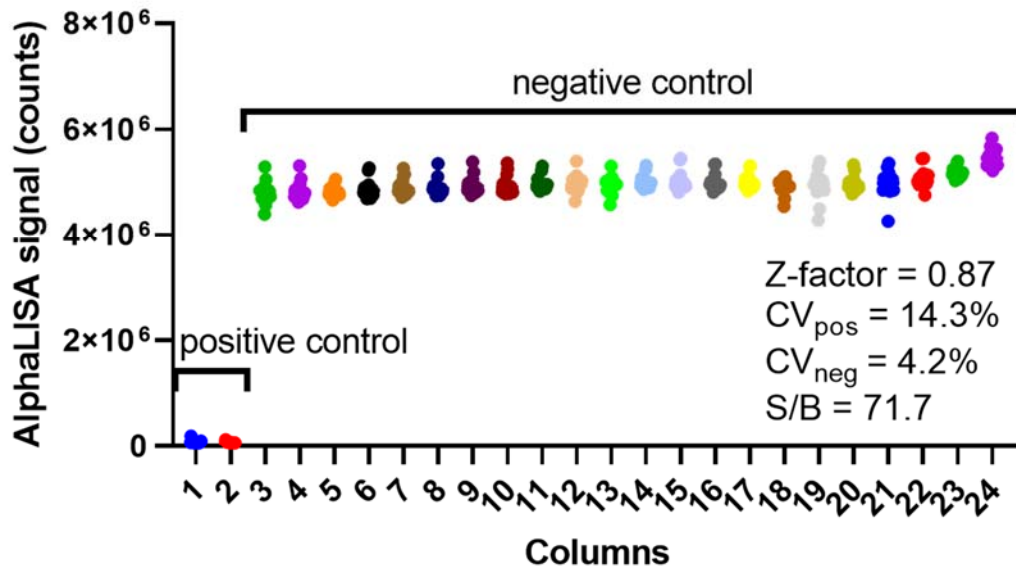
Lysate Dilution Table	
Fold dilution	Number of Cells
1/1	450,000
1/15	30000
1/30	15000
1/60	7500
1/120	3750
1/240	1875
1/480	938
1/960	469
1/1920	234

D

Interpolated Concentration (ng/mL)	
24 hr Lysate	7,300
48 hr Lysate	34,092

Figure S-10 Estimation of viral NP concentration in viral lysates. **(A)** Detection of titrated 0 hr, 24 hr, and 48 hr lysates with NCATS LB using pair 11+4. **(B)** S/B calculation from graph in A. Red values indicate linear range of the curve. **(C)** Interpolated concentrations of NP in 24 hr and 48 hr viral cell lysates. **(D)** Interpolated NP (ng/mL) from samples in A. N = duplicate wells. Error bars indicate S.D.

A



B

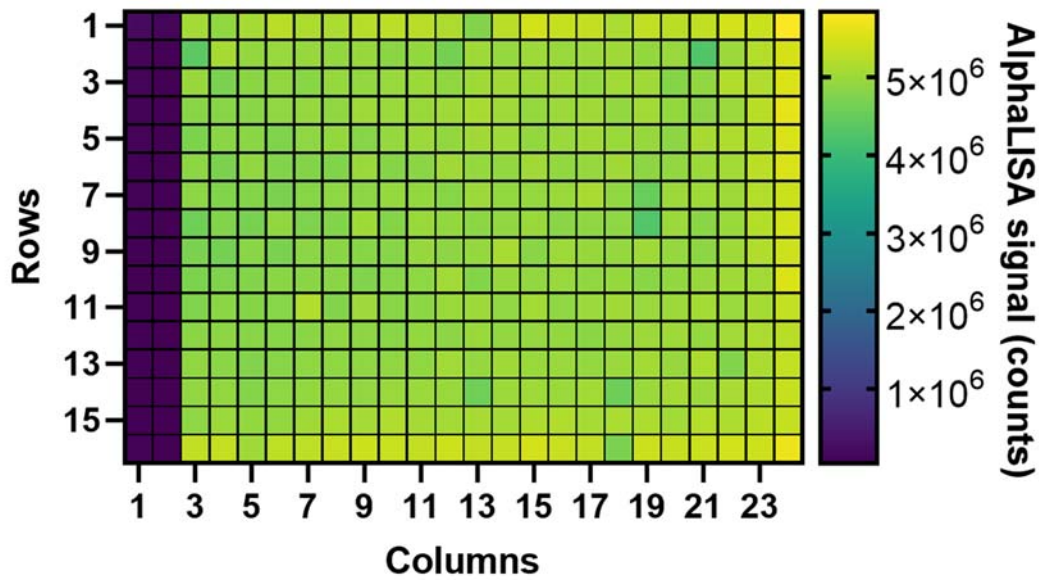


Figure S-11 Stability of the assay after 24 hours. **(A)** Graph illustrating the AlphaLISA signal counts per well in each column and the calculated plate statistics for a 384-well plate using a total volume of 16 μ L. The positive control is media only and the negative control is media with 500 ng/mL recombinant NP. **(B)** Heat map demonstrating the AlphaLISA signal counts of each well.