Supplementary Material for:

Direct Immunoassay on Polyester Microwell Plate for Colorimetric Detection of Spike Protein in Swab and Saliva Samples

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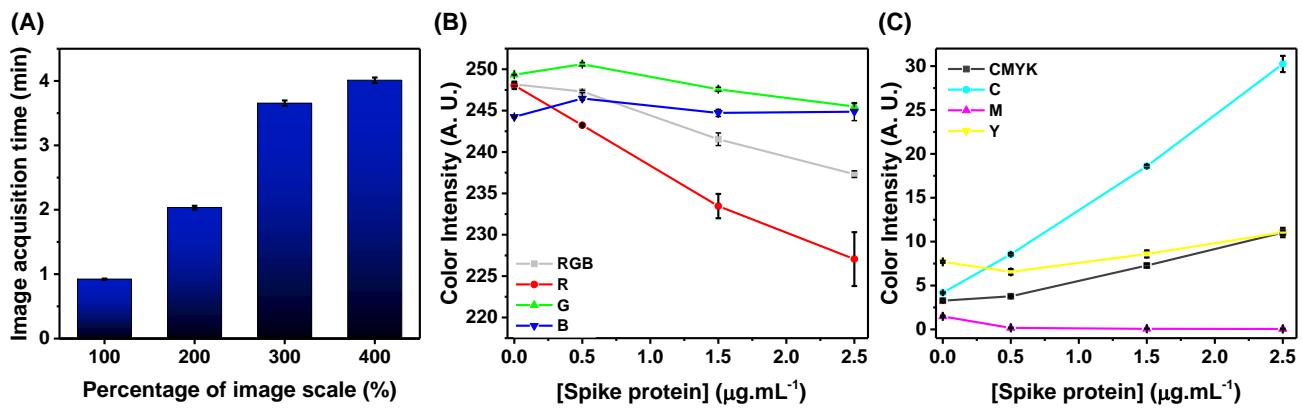
Figure S1. (A) Evaluation of the image percentage scale in image acquisition time using the scanner. Colorimetric responses obtained from the S protein detection reaction on the microplate were analyzed in the color systems RGB (B) and CMYK (C) of different color channels.
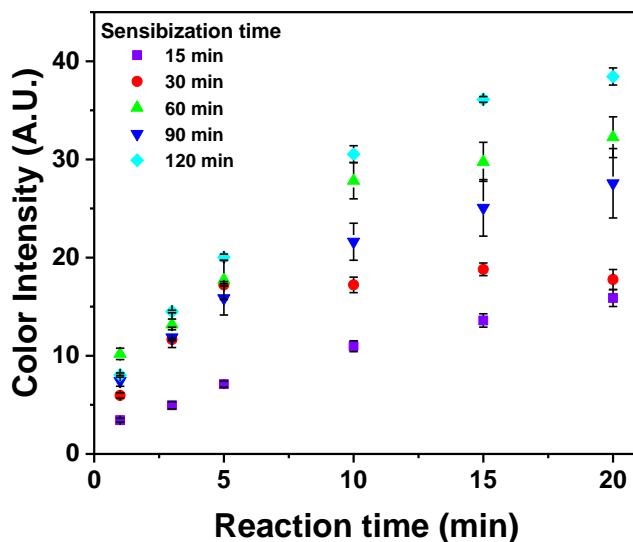
Figure S2. Optimization of sensitization step of direct ELISA for detection of SARS-CoV-2. The concentration of S-protein used was 1.5 µg mL^{-1}.

Table S1. Evaluation of repeatability intraplate and interplate.

[S protein] µg. mL^{-1} (n=5)	RSD (%)	
	Intraplate (1 plate)	**Interplate (3 plates)**
1.5	3.7	7.7
2.5	7.2	5.9
5.0	7.8	2.3
10.0	3.7	5.2

Table S2: Results of human Saliva and Swab samples tested using the gold standard RT-qPCR assay and the developed direct ELISA method on a polyester plate.

Sample	Sample type	Classification gold std.	direct ELISA					
			1st	2nd	3rd	Average (A.U.)	Std dev.	Classification
1	Saliva	Negative	4.80	5.10	4.48	**4.79**	**0.31**	Negative
2	Saliva	Negative	4.23	4.06	4.35	**4.21**	**0.15**	Negative
3	Saliva	Negative	5.21	5.1	5.32	**5.21**	**0.11**	Negative
4	Saliva	Negative	3.24	3.05	3.75	**3.35**	**0.36**	Negative
5	Saliva	Negative	4.31	4.48	5.00	**4.60**	**0.36**	Negative
6	Saliva	Negative	5.20	5.33	5.34	**5.29**	**0.08**	Negative
7	Saliva	Negative	6.23	6.49	5.48	**6.07**	**0.52**	Positive
8	Swab	Negative	4.76	3.49	3.84	**4.03**	**0.66**	Negative
9	Swab	Negative	4.30	4.45	4.10	**4.28**	**0.18**	Negative
10	Swab	Negative	3.16	3.44	3.1	**3.23**	**0.18**	Negative
11	Swab	Negative	5.42	5.24	4.47	**5.04**	**0.50**	Negative
12	Swab	Negative	3.28	3.08	3.32	**3.23**	**0.13**	Negative
13	Swab	Negative	2.57	2.56	2.74	**2.62**	**0.10**	Negative
14	Swab	Negative	4.31	4.84	4.69	**4.61**	**0.27**	Negative
15	Swab	Negative	4.23	4.62	4.52	**4.46**	**0.20**	Negative
16	Swab	Negative	3.24	3.40	4.32	**3.65**	**0.58**	Negative
17	Saliva	Positive	6.23	6.43	6.34	**6.33**	**0.10**	Positive
18	Saliva	Positive	6.70	7.20	6.19	**6.70**	**0.51**	Positive
19	Saliva	Positive	6.06	6.58	6.79	**6.48**	**0.38**	Positive
20	Saliva	Positive	6.24	6.94	6.6	**6.59**	**0.35**	Positive
21	Saliva	Positive	6.37	6.96	7.36	**6.90**	**0.50**	Positive
22	Saliva	Positive	6.72	6.92	6.82	**6.82**	**0.10**	Positive
23	Saliva	Positive	10.04	9.37	10.76	**10.06**	**0.70**	Positive
24	Saliva	Positive	18.70	17.08	17.61	**17.80**	**0.83**	Positive
25	Saliva	Positive	14.73	13.45	14.07	**14.08**	**0.64**	Positive
26	Saliva	Positive	13.64	12.42	12.94	**13.00**	**0.61**	Positive
27	Saliva	Positive	9.92	8.56	9.23	**9.24**	**0.68**	Positive
28	Swab	Positive	9.84	9.12	10.21	**9.72**	**0.55**	Positive
29	Swab	Positive	7.22	7.17	7.24	**7.21**	**0.04**	Positive
30	Swab	Positive	6.22	6.36	6.10	**6.23**	**0.13**	Positive
31	Swab	Positive	22.73	20.96	21.93	**21.87**	**0.89**	Positive
32	Swab	Positive	9.57	8.36	9.09	**9.01**	**0.61**	Positive
33	Swab	Positive	9.81	9.13	10.94	**9.96**	**0.91**	Positive
34	Swab	Positive	7.28	6.83	6.35	**6.82**	**0.47**	Positive
35	Swab	Positive	7.01	7.97	7.34	**7.44**	**0.49**	Positive
36	Swab	Positive	4.61	4.15	4.75	**4.50**	**0.31**	Negative
37	Swab	Positive	6.28	6.51	6.35	**6.38**	**0.12**	Positive

Table S3. Cost estimate for microwell plate immunoassay on polyester substrates

Consumables	Quantity	Cost (US$)	Manufactured plate quantity	Total cost/ plate (US$)
Thermal laminating Sheets (A4)	1 unit	0.53	2.67	0.20
Plate total				**0.20**

Reagents	Concentration	Vol./microwell (μL)	Cost (US$)/microwell	Total cost (US$)/ plate
Blocking solution	1% (v:v)	2	1.41×10^{-4}	0.013
Biotinylated detection antibody	2.0 (μg. mL^{-1})	2	1.39×10^{-2}	1.33
Streptavidin-HRP	1:1000 (v:v)	2	1.25×10^{-3}	0.12
TMB Liquid Substrate, for ELISA	-	2	2.16×10^{-3}	0.21
Step washing	10 mmol.L^{-1} PBS and 0.05% Tween 20	Volume/step - 10 mL	2.67×10^{-2}	0.11
Total reagents				**1.78**
Total cost (US$)/ plate immunoassay				**1.98**
Total cost (US$)/ microwell				**0.021**