

Table S1. Effects of λ_1 and λ_2 on R_{12} and R_{21}

Mapada	R_{12}	λ_1 and λ_2 (nm)				R_{21}	λ_1 and λ_2 (nm)			
	R_{12}	405/450	405/460	400/450	400/460		405/450	400/450	405/460	400/460
	Mean	0.0820	0.0772	0.0053	0.0050	mean	0.1867	0.1840	0.0832	0.0820
	SD	0.0040	0.0038	0.0027	0.0025	SD	0.0049	0.0049	0.0052	0.0052
Biotek	R_{12}	405/450				R_{21}	450/405			
	mean	0.0818	nd	nd	nd	mean	0.1917	nd	nd	nd
	SD	0.0019	nd	nd	nd	SD	0.0022	nd	nd	nd

nd: no determination.

Table S2 Comparison of LOD, LOQ by SDESA versus those by separate assay on Biotek ELX800 microplate reader

Conditions	$(n=7)$	Wavelength	450 nm		405 nm	
		Methods	SDESA	Separate assay	SDESA	Separate assay
absorbance increase in 40 min	System Fluctuation	mean	0.003	0.003	0.002	0.001
		SD	0.002	0.002	0.001	0.001
		LOD	0.009	0.009	0.005	0.005
absorbance increase in 40 min	BGAL:AGLU (1:1)	STEYX	0.006	0.007	0.005	0.004
		LOQ	0.039	0.045	0.029	0.026
absorbance increase in 40 min	BGAL:AGLU (1:3)	STEYX	0.007	0.006	nd	nd
		LOQ	0.042	0.039	nd	nd
absorbance increase in 40 min	BGAL:AGLU (1:27)	STEYX	0.009	0.008	nd	nd
		LOQ	0.054	0.051	nd	nd
absorbance increase in 40 min	BGAL:AGLU (3:1)	STEYX	nd	nd	0.006	0.005
		LOQ	nd	nd	0.037	0.032
absorbance increase in 40 min	BGAL:AGLU (27:1)	STEYX	nd	nd	0.009	0.008
		LOQ	nd	nd	0.048	0.044

BGAL: β -D-galactosidase; AGLU: α -D-glucosidase; STEYX: standard error of estimate; nd: no determination.

Table S3. LODs of two analytes by ELISA

B/B ₀ (%) (n=5)		mean	SD	mean-3*SD	LOD (ng/well)
SDESA	Penicillin G	100.0	2.6	92.3	1.7
	Clenbuterol	100.0	1.9	94.4	1.3
Separate assay	Penicillin G	100.0	1.3	96.2	1.1
	Clenbuterol	100.0	1.7	94.8	1.3

Table S4 Consistency of results by ELISA via SDESA with that via separate assay

Sample NO.	Penicillin G (n=5)					Clenbuterol (n=5)				
	SDESA-ELISA B/B ₀ (%)	Separate assay B/B ₀ (%)	SDESA-ELISA (ng/well)	Separate assay (ng/well)	consistency (%)	SDESA-ELISA B/B ₀ (%)	Separate assay B/B ₀ (%)	SDESA-ELISA (ng/well)	Separate assay (ng/well)	consistency (%)
mean					99					99
SD					6					8
1	48.60	46.00	355	391	91	46.26	49.13	52	56	92
2	68.86	67.73	23	24	94	82.23	79.26	nd	nd	nd
3	46.47	44.56	474	469	101	63.97	66.33	12	13	89
4	44.59	41.92	612	657	93	31.83	35.11	171	183	94
5	52.34	51.17	214	202	106	43.80	46.66	63	69	91
6	79.63	79.73	nd	nd	nd	30.41	34.79	193	188	103
7	25.65	20.90	nd	nd	nd	86.40	88.74	nd	nd	nd
8	61.66	61.06	60	57	106	100.93	98.77	nd	nd	nd
9	59.19	56.78	85	99	86	27.64	32.20	243	234	104
10	43.20	41.71	738	676	109	34.67	37.44	135	151	90
11	85.94	86.14	nd	nd	nd	35.76	39.80	124	124	100
12	45.47	43.08	543	567	96	41.81	46.50	75	70	106
13	43.67	40.94	693	745	93	32.14	35.42	167	179	94
14	50.12	48.96	289	268	108	40.12	43.96	86	87	99
15	44.03	42.12	660	641	103	47.58	52.29	46	43	107
16	54.14	52.29	168	175	96	99.95	104.18	nd	nd	nd
17	64.23	63.67	43	41	104	32.94	37.56	156	149	105
18	50.40	49.01	278	266	105	37.40	41.99	108	103	105
19	55.41	53.60	141	148	95	41.07	43.10	80	94	85
20	31.00	31.05	nd	nd	nd	87.78	93.15	nd	nd	nd
21	47.67	45.56	403	413	97	38.76	43.96	96	87	111
22	81.22	82.16	nd	nd	nd	25.49	29.69	nd	nd	nd
23	44.50	42.35	619	622	99	29.07	32.90	216	221	98
24	45.81	43.49	518	538	96	36.82	41.95	113	103	110
25	67.53	67.32	27	26	106	72.04	76.11	6	6	103
26	57.85	55.97	101	109	93	51.35	54.41	34	36	93
27	86.23	80.04	nd	nd	nd	90.76	82.66	nd	nd	nd

Only those data within quantifiable ranges were processed. nd: no determination. Samples with random unknown quantities of two analytes were used to test consistency of ELISA by separate assay and SDESA.

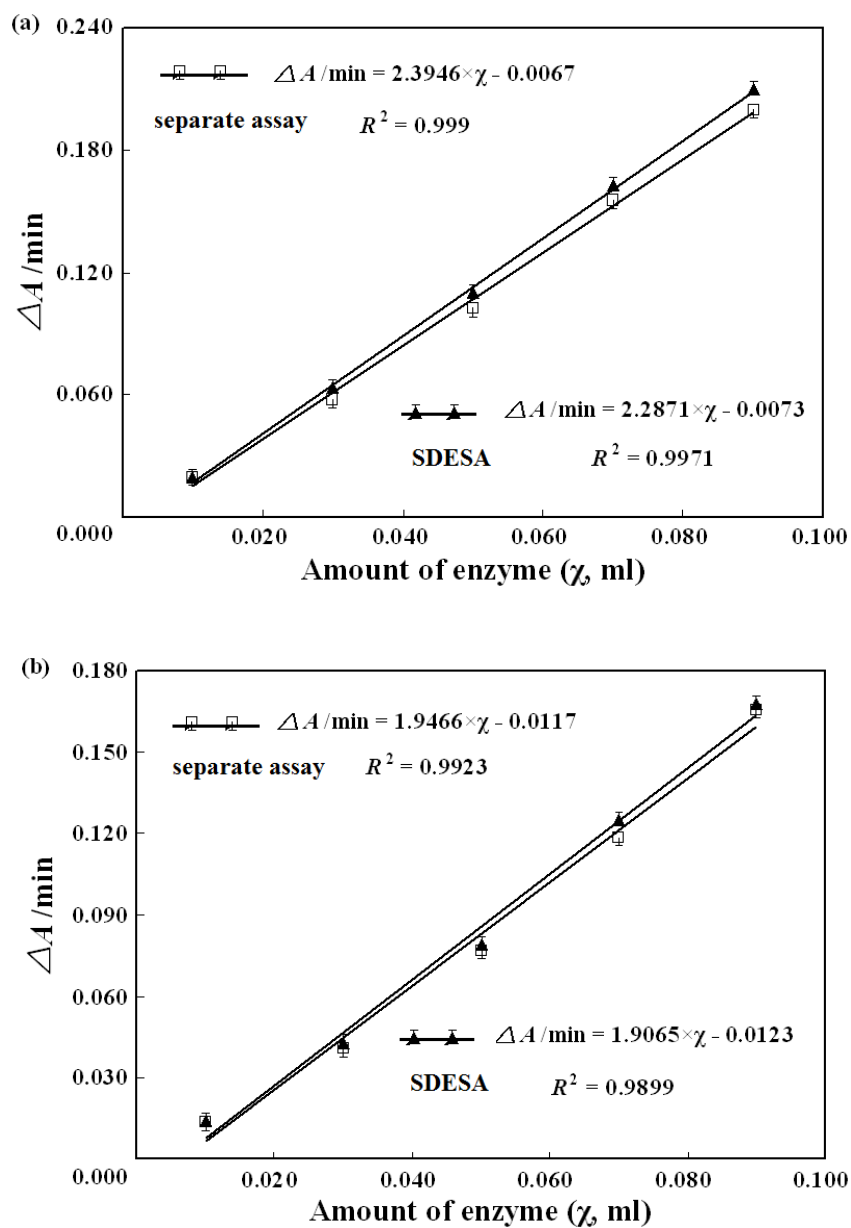


Figure S1 Response curves of β -D-galactosidase and α -D-glucosidase by SDESA and separate assay

(a) β -D-galactosidase; (b) α -D-glucosidase;