Electronic Supplementary Material (ESI) for Analyst.

Supplementary Materials

Simultaneous detection of carcinoembryonic antigen and neuron specific enolase in human serum based on time-resolved chemiluminescence immunoassay

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Contents:

S-1 Buffer solution

The buffer solution used in the experiment were as follows: coated buffer 0.10 mol L^{-1} Tris-HCl (pH 8.5), 0.01 mol L^{-1} PBS (pH 7.4), 0.01 mol L^{-1} Tris-HCl (pH 10.2), 0.01 mol L^{-1} MES (pH 6.0), PBST (0.01 mol L^{-1} PBS containing 0.05% Tween-20, pH 7.4), PBS/BSA (0.01 mol L^{-1} PBS containing 0.5% BSA, pH 7.4).

S-2. Optimization of the concentration luminol, H_2O_2 and BIP.

As shown in the Fig. S1A, chemiluminescence intensity (RLU) was the highest when the concentration of luminol was 1.4 mmol L^{-1}. Therefore, 1.4 mmol L^{-1} luminol was selected for further study. In the same way, 3.0 mmol L^{-1} H_2O_2 (Fig. S1B) and 7.5 × 10^{-2} mmol L^{-1} BIP were used in further investigations (Fig.S1C).

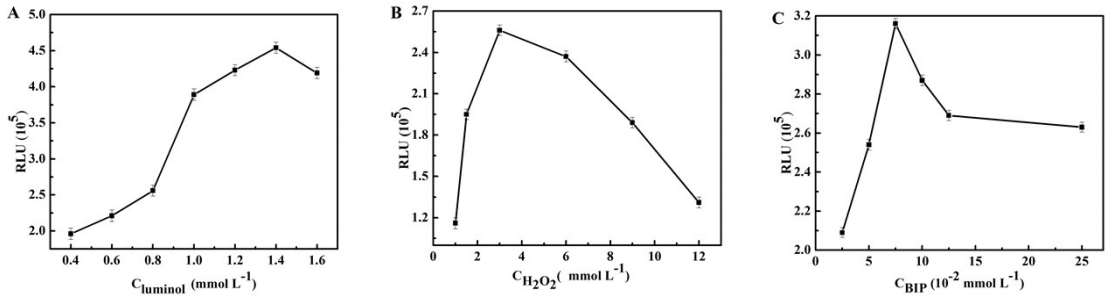

Fig. S1. Single factor optimization of the HRP-luminol-H_2O_2-BIP system under different concentration of luminol (A), H_2O_2 (B) and BIP (C).

S-3. Optimization the concentration of AMPPD.

The influence of AMPPD of different concentration on CL signals is shown in the Fig. S2. Within the concentration range of 5.0-25 mmol L^{-1}, RLU increased with the increase of AMPPD concentration. When the concentration exceeded 25 mmol L^{-1}, RLU was basically unchanged. Therefore, 25 mmol L^{-1} was selected as the optimal concentration of AMPPD.

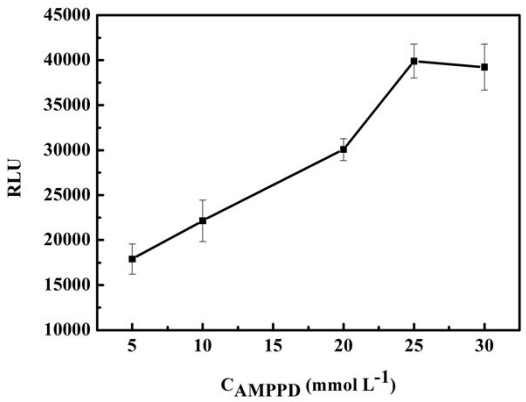

Fig. S2. Optimization of ALP-AMPPD system under different concentrations of AMPPD

S-4. Selection of coated buffer.

In this experiment, PBS (0.01 mol L^{-1} pH 7.2), carbonic acid buffer (CB) (0.05 mol L^{-1}, pH 9.6), Tris-HCl (0.1 mol L^{-1}, pH 8.5) and Tris-HCl (0.01 mol L^{-1}, pH 10.2) were selected for investigation. As can be seen from Fig.S3, HRP and ALP systems had weak signal-to-noise ratio in PBS. In CB and Tris-HCl (pH 10.2), the signal-to-noise ratios of HRP system were significantly different from those of ALP system. Only Tris-HCl (pH 8.5) was more compatible with the mixed system. Therefore, Tris-HCl (pH 8.5) was finally selected as the coated buffer.

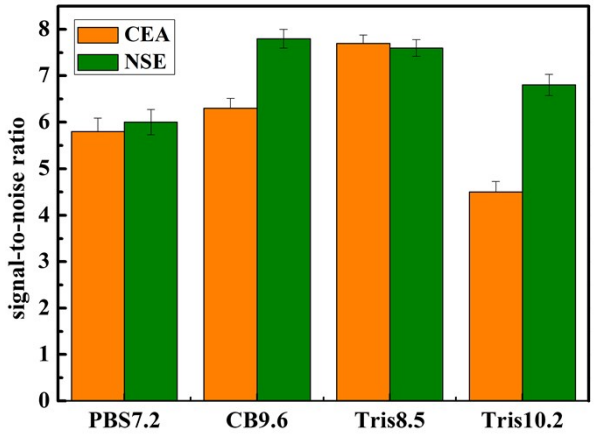

Fig.S3. Influence of the CEA mAbs@CPSMS or NSE mAbs@CPSMS coated buffer on the signal-blank-ratio for CEA and NSE detections.

S-5. Detailed results detected by TRCLIA and CLEIA kits.

Table S1 Detailed results detected by TRCLIA and CLEIA kits.

Serum samples	$CEA_{CLEIA\ kit}$ (ng mL^{-1})	CEA_{TRCLIA} (ng mL^{-1})	$NSE_{CLEIA\ kit}$ (ng mL^{-1})	NSE_{TRCLIA} (ng mL^{-1})	Serum samples	$CEA_{CLEIA\ kit}$ (ng mL^{-1})	CEA_{TRCLIA} (ng mL^{-1})	$NSE_{CLEIA\ kit}$ (ng mL^{-1})	NSE_{TRCLIA} (ng mL^{-1})
1	0.95	1.12	29.21	25.18	24	1.79	1.68	35.09	36.78
2	1.94	1.69	37.61	41.92	25	1.59	1.33	17.74	19.25
3	0.88	0.95	51.57	47.54	26	2.15	2.03	54.91	56.31
4	2.09	2.28	28.97	26.47	27	3.72	3.55	40.61	38.86
5	3.43	3.31	36.27	39.03	28	2.59	2.67	47.96	49.07
6	5.08	5.31	25.30	23.17	29	3.26	3.11	69.73	73.03
7	2.75	2.89	9.23	11.97	30	1.05	0.89	34.79	35.19
8	3.25	3.14	34.86	30.79	31	0.01	0.23	25.41	26.38
9	2.25	2.18	46.35	49.39	32	0.81	0.88	53.58	50.78
10	1.32	1.23	23.61	20.88	33	2.82	2.93	41.96	41.24
11	0.64	0.45	44.04	47.25	34	2.15	2.04	17.36	18.03
12	0.81	0.69	43.16	40.15	35	0.65	0.77	36.22	35.07
13	0.71	0.58	56.83	51.53	36	3.43	3.29	21.15	22.71
14	0.74	0.86	53.11	50.61	37	2.02	2.25	28.62	27.00
15	3.00	3.17	47.63	49.43	38	1.21	1.09	33.82	35.14
16	1.15	1.23	95.30	109.20	39	1.46	1.34	32.91	31.01
17	2.40	2.34	47.38	51.15	40	1.33	1.42	7.88	8.46
18	5.68	5.41	108.10	96.80	41	0.62	0.75	6.96	7.11
19	0.47	0.60	35.07	37.49	42	1.39	1.50	78.71	82.96
20	2.38	2.54	51.30	54.80	43	3.29	3.41	17.95	14.68
21	2.33	2.25	80.08	75.09	44	2.06	1.95	54.42	58.93
22	3.22	3.11	81.53	86.47	45	1.10	1.21	40.60	43.10
23	1.46	1.59	25.78	24.15					

S-6. Comparison of the proposed method with other methods.

Table S2 Comparison of the proposed method with other methods.

Methods	*RSD* (%)		limits of detection (ng mL^{-1})		Refs
	CEA	NSE	CEA	NSE	
Immunomagnetic Nanobeads/lateral flow test strip	2.5-5.2	2.7-5.9	0.045	0.094	1
Fluorescence quantum dots	0.53	0.53	1.0	1.0	2
Microarray/gold nanoparticles	—	—	0.75	0.98	3
Fluorescence immunoassay	2.23-5.13	0.09-6.36	0.625	0.625	4
AuNPs/tryptophan and caffeic acid-based resin microspheres	5.9	7.9	0.11	0.08	5
Time-resolved chemiluminescence immunoassay	**4.8-9.0**	**2.3-4.3**	**0.085**	**0.044**	**This work**

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