Electronic supplementary information

1. Buffers and solutions

The sodium carbonate coating buffer consisted of 1.59 g Na_2CO_3 and 2.93 g $NaHCO_3$ (pH 9.6). The phosphate-buffered saline solution (PBS, pH 7.4) consisted of 0.22 g KH_2PO_4, 2.9 g $Na_2HPO_4 \cdot 12H_2O$, 0.20 g KCl and 8.0 g NaCl. The PBS wash buffer (PBST) was composed of 0.05% (v/v) Tween-20. The TMB substrate solution was prepared by adding 1mg of TMB to 400 μL of ethanol followed by 10 mL of phosphate-citrate buffer (0.1 mol L^{-1} citric acid and 0.2 mol L^{-1} Na_2HPO_4, pH 4.3) containing 10 μL of a 30% H_2O_2 solution.

2. Preparation of protein-hapten conjugates

A modified active ester method was used to conjugate hapten with BSA. The hapten (64 mg) was dissolved in 0.4 ml N, N-dimethylformamide (DMF), and a solution of NHS (21.4 mg) and DCC (40.7 mg) in DMF (0.5 mL) was then added to the hapten solution. This solution was magnetically stirred for 8 h at room temperature. The supernatant liquor from the hapten solution was added to a BSA solution that was prepared by dissolving 82 mg of BSA in 5 mL of PBS with stirring. The BSA mixture was stirred at 4 °C for 6 h before being dialysed (molecular weight cut-off 6000 Da) against three changes of PBS per day for 3 days at 4 °C. After sediment removal by centrifugation, the obtained conjugate was stored at -20 °C.

The hapten was coupled to OVA by a modified mixed anhydride method to produce a coating antigen. The hapten (32.2 mg) was dissolved in DMF (0.5 mL)

prior to adding n-butylamine (30 μL) and isobutyl chloroformate (20 μL). The reaction to produce the mixed anhydride was allowed to proceed for 1h with stirring at 4 °C. The mixed anhydride solution was then added drop-wise to the OVA solution (prepared by dissolving 50 mg of OVA in 5 mL of PBS with thorough stirring). The mixture was stirred for 4 h and then dialysed in PBS for 3 days at 4 °C and then stored at -20 °C.

3. Sample preparation

Each sample was immersed in 20 mL of an acetone / n-hexane mixture (v/v=1:1) and placed in an ultrasonic bath for 25 min three separate times. The resulting supernatant was concentrated down to 3 mL using a rotary evaporator. The concentrated mixture was then cleaned with 1 mL concentrated sulphuric acid to clean away lipoid and other organic compounds, and then run through a column filled with anhydrous sodium sulphate and Florisil. Normal hexane was used as prewash solvent and elution solvent. The final solution was concentrated and divided into two fractions: one for the ELISA detection and the other for GC- ECD analysis.

The treated samples for ELISA detection were dried by nitrogen and diluted to the proper concentrations with PBSTM (PBST with 5% DMSO). The other fraction for GC- ECD analysis was diluted with pesticide-grade hexane.

4. GC-ECD analysis

GC-ECD analysis was performed to evaluate the accuracy of the ic-ELISA using a SPB^{TM}-5 fused silica capillary column (30 m-0.25 mm; 0.25 μm ®lm thickness) obtained from Agilent, a μ-ECD detector, and nitrogen as the carrier gas. The injector

temperature was set to 250 °C and the detector temperature at 310 °C. The initial GC temperature was programmed to 100 °C for 2 min, then to 230 °C at 10 °C min^{-1} for 2 min. The temperature was then increased from 230 °C to 290 °C at 5 °C min^{-1}.

5. *Identification of Conjugates*

UV spectra were employed to monitor the effectiveness of the conjugation reaction. The spectrum of the hapten–BSA conjugate showed a wider absorption band than the spectrum of BSA, and both have different spectra compared with the spectrum of hapten (Fig. S1). The spectrum of the coating antigen shows a wider absorption band than the spectrum of OVA, and both have different spectra compared with the hapten spectrum (Fig. S2). The results show that the hapten and the carrier protein (BSA and OVA) have been coupled successfully

57 Fig. S1. UV spectra of hapten PCB12, hapten- BSA and BSA.

58 Fig. S2. UV spectra of hapten PCB12, hapten-OVA and OVA.

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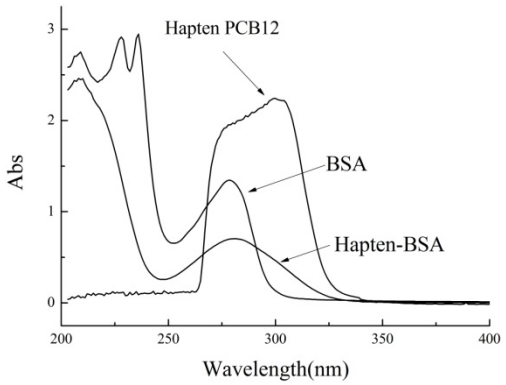

Fig. S1. UV spectra of hapten PCB12, hapten- BSA and BSA.

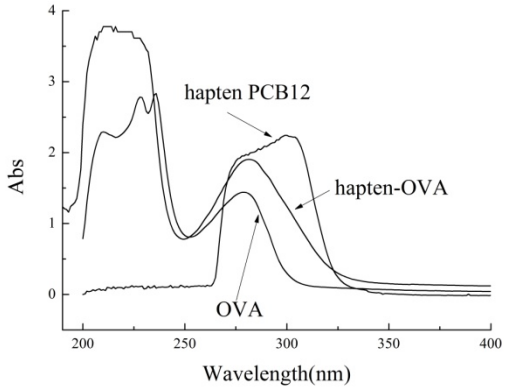

Fig. S2. UV spectra of hapten PCB12, hapten-OVA and OVA.

Table S1. Effect of the first incubation time on the ELISA Sensitivity.

First incubation time (min)	A_{max}	IC_{50} ($\mu g\ L^{-1}$)
Overnight (4°C)	1.15	2.32
60 (37°C)	0.86	3.12
90 (37°C)	0.94	3.19
120 (37°C)	1.04	2.85

Table S2. Effect of the second incubation time on the ELISA Sensitivity.

Second incubation time (min)	A_{max}	IC_{50} ($\mu g\ L^{-1}$)
30	0.89	2.56
60	1.13	2.39
90	1.18	2.61
120	1.29	3.11

Table S3. Effect of the organic solvent on the ELISA Sensitivity.

DMSO (V/V, %)	A_{max}	IC_{50} ($\mu g\ L^{-1}$)
5	1.09	2.55
10	0.87	2.78
15	0.84	3.68
20	0.81	3.91

Table S4. Effect of the pH value on the ELISA Sensitivity.

pH	A_{max}	IC_{50} ($\mu g\ L^{-1}$)
6.5	0.94	2.76
7.0	1.02	2.69
7.4	1.02	2.51
8.0	0.96	2.81
8.5	0.92	3.01

Table S5. Effect of ionic strength on the ELISA Sensitivity.

ionic strength (mol L^{-1})	Amax	IC_{50} ($\mu g\ L^{-1}$)
0.10	1.03	2.49
0.14	1.06	2.41
0.20	1.05	2.62
0.30	0.92	3.12
0.40	0.89	3.34