

# Development of Ic-ELISA and Colloidal Gold Lateral Flow Immunoassay for the Determination of Cypermethrin in Agricultural Samples

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## Part S1. The checkerboard procedure of ic-ELISA

To optimize the concentrations of the coated antigen and antibody of ic-ELISA, checkerboard titration was carried out. Briefly, the coating antigen was diluted into a series of concentrations (2, 1, 0.5, 0.25 and 0.125 µg/mL) and coated five row wells of microplates. After the plates were blocked, the antibody was diluted into serial concentrations (2, 1, 0.5, 0.25 and 0.125 µg/mL) and added into the coated wells column by column. The following steps were the same as those used in ic-ELISA procedure. The concentration of antibody and coating antigen of the well, which of the OD value is about 1.0, are determined as the optimal working condition.

**Table S1.** The hapten-to-protein molar ratios of conjugates.

Conjugates	Coupling ratio	Conjugates	Coupling ratio
H1-KLH	12.1:1	H4-BSA	17.1:1
H1-BSA	3.6:1	H5-BSA	1.3:1
H2-BSA	14.9:1	H6-BSA	6.5:1
H3-BSA	2.6:1	H7-BSA	2.0:1

**Table S2.** Sensitivities of anti-CYP antibodies using hapten 1 as coating antigen.

Compound	Cell line	IC <sub>50</sub> (ng/mL)
Cypermethrin	2G7H9	19
	2G9A8	176
	1E7D5	314
	6F1G10	575

**Table S3.** Effect of of sodium ion concentration, pH, type and content of organic solvents on sensitivity of ic-ELISA for determination of CYP.

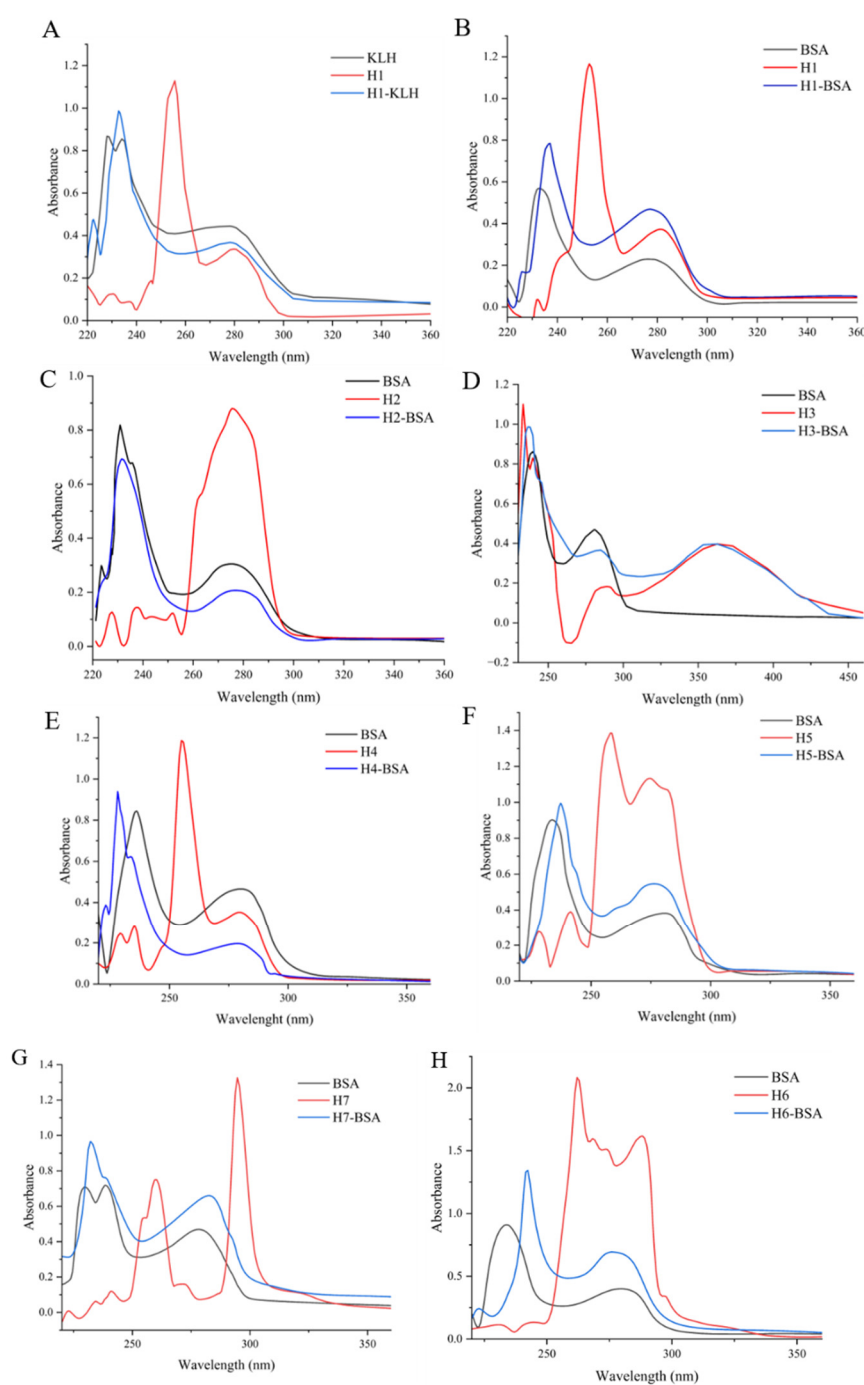
Working buffer conditions		A <sub>max</sub>	IC <sub>50</sub> (ng/mL)	A <sub>max</sub> /IC <sub>50</sub>
10% Methanol		1.57	7.67	0.20
10% Acetonitrile		1.15	8.88	0.13
10% Acetone		1.25	7.99	0.17
10% Dimethyl sulfoxide		1.38	11.03	0.13
Methanol	5%	1.51	30.63	0.05
	10%	1.37	8.60	0.17
	20%	0.99	20.59	0.05

	40%	0.65	24.47	0.03
	0.07	2.40	43.31	0.06
	0.14	2.31	9.22	0.25
Na <sup>+</sup> (mol/L)	0.2	1.80	13.58	0.13
	0.3	1.32	2.42	0.55
	0.4	0.65	2.94	0.22
	6	0.81	4.41	0.18
	7	1.17	5.24	0.22
pH	7.4	1.24	3.09	0.40
	8	1.10	8.06	0.14
	9	0.70	4.03	0.17

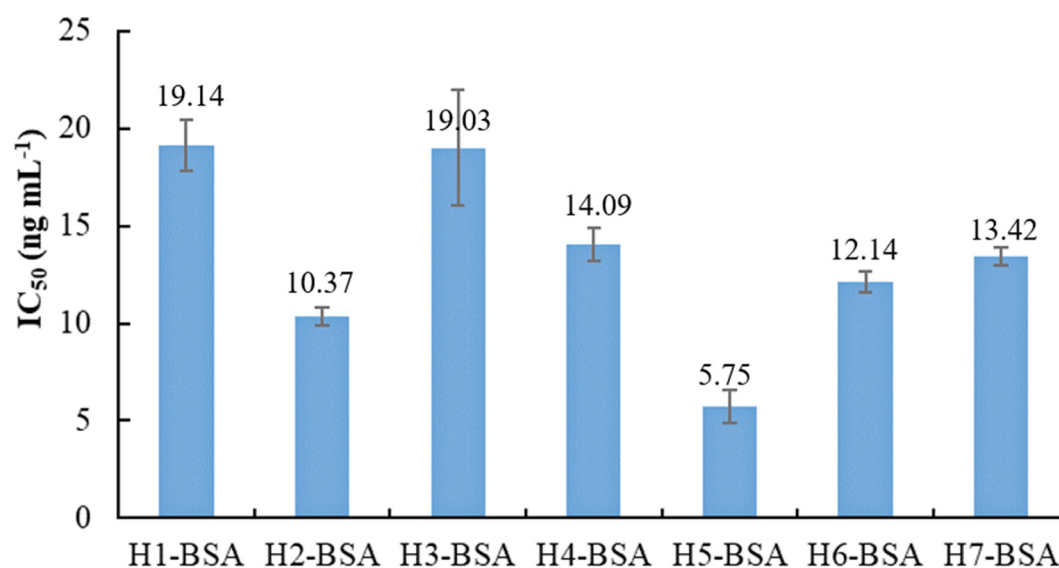
**Table S4.** Influence of sodium ion concentration, pH, type and content of organic solvents on sensitivity of LFIA for determination of CYP.

Working buffer conditions		0 µg/mL	0.3 µg/mL	0.4 µg/mL	0.6 µg/mL
Na <sup>+</sup> (mol/L)	0.07	+++ <sup>a</sup>	+++	++-	---
	0.14	+++	+++	++-	+-
	0.25	+++	+++	++-	+-
	0.5	++-	++-	++-	+-
	1	++-	++-	++-	+-
	2	+-	+-	+-	---
pH	6	+++	+++	++-	---
	7	+++	+++	+-	---
	7.4	+++	+++	++-	---
	8	++-	++-	+-	---
	9	+-	+-	---	---
Organic solvents (v%)	Methanol	10	+++	++-	---
		20	+++	++-	+-
		30	++-	++-	+-
		40	+-	++-	---
	Acetonitrile	10	+++	++-	+-
		20	++-	++-	---
		30	+-	++-	---
		40	---	---	---
	Acetone	10	+++	++-	+-
		20	+++	++-	+-
		30	++-	++-	---
		40	+-	---	---
	Dimethyl sulfoxide	10	+++	++-	---
		20	+++	++-	---
		30	++-	++-	---
		40	++-	++-	---

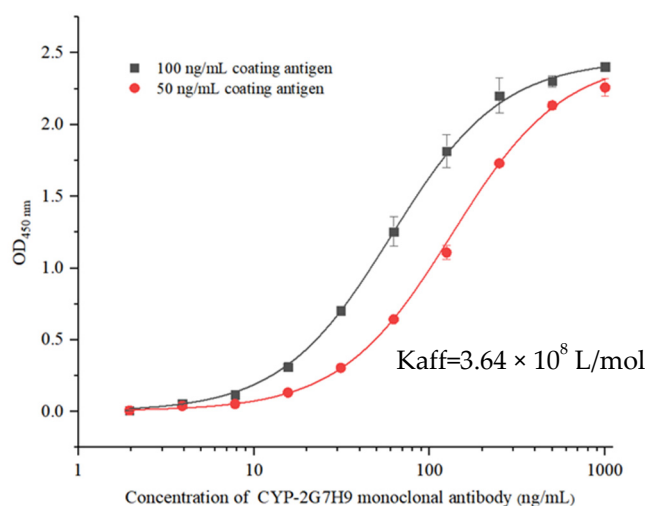
a: More plus signs (+) mean darker red; --- means no color band.



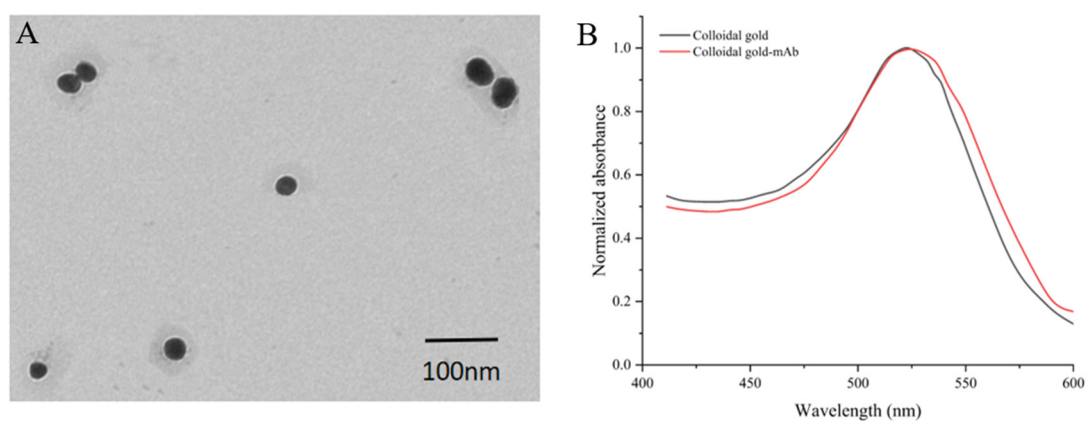
**Figure S1.** The UV-Vis spectra of hapten-carrier protein conjugates. (A) Hapten 1-KLH; (B) Hapten 1-BSA; (C) Hapten 2-BSA; (D) Hapten 3-BSA; (E) Hapten 4-BSA; (F) Hapten 5-BSA; (G) Hapten 6-BSA; (H) Hapten 7-BSA.



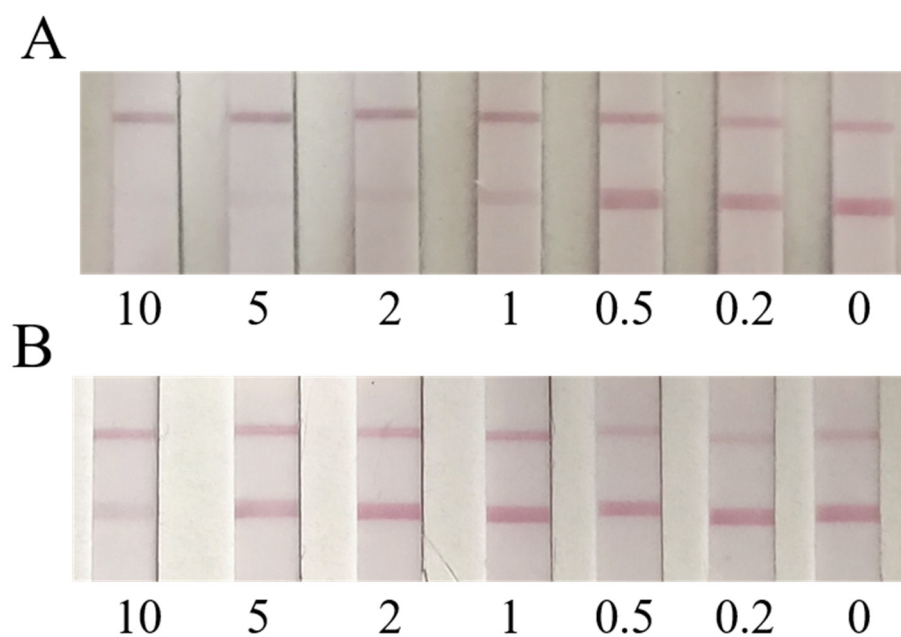
**Figure S2.** The sensitivity of mAb 2G7H9 using different coating antigens.



**Figure S3.** The K<sub>aff</sub> value of mAb 2G7H9.



**Figure S4.** Identification of colloidal gold and colloidal gold-mAb. (A) The transmission electron micrograph of the colloidal gold; (B) The UV-Vis spectra of colloidal gold and colloidal gold-mAb.



**Figure S5.** The vLOD of LFIA for cyfluthrin (A) and cyhalothrin (B) determination, the concentrations from left to right were 10, 5, 2, 1, 0.5, 0.2 and 0  $\mu\text{g/mL}$ .