

## *Supporting information*

### **Hapten Synthesis and a Colloidal Gold**

### **Immunochromatographic Strip Assay to Detect Nitrofen and Bifenox in Fruits**

Peng Wang<sup>1,2,3</sup>, Xinxin Xu<sup>1,2,3</sup>, Lingling Guo<sup>1,2,3</sup>, Liqiang Liu<sup>1,2,3</sup>, Hua Kuang<sup>1,2,3</sup>, Jing Xiao<sup>b\*</sup>,  
Chuanlai Xu<sup>1,2,3\*</sup>

<sup>1</sup>State Key Laboratory of Food Science and Technology, Jiangnan University; <sup>2</sup>International Joint Research Laboratory for Biointerface and Biodetection and School of Food Science and Technology, Jiangnan University; <sup>3</sup>Collaborative Innovation center of Food Safety and Quality Control in Jiangsu Province, Jiangnan University, Wuxi, Jiangsu, 214122, People's Republic of China; <sup>4</sup>NHC Key Laboratory of Food Safety Risk Assessment, China National Center for Food Safety Risk Assessment, Beijing, People's Republic of China;

\*Corresponding author, Email: [xiaojing@cfsa.net.cn](mailto:xiaojing@cfsa.net.cn); [xcl@jiangnan.edu.cn](mailto:xcl@jiangnan.edu.cn);

**Fig. S1** Composition of the test strip (a) and the schematic for samples detection (b).

**Fig. S2** The mass spectrum of NIT-1. (a) The total ion current spectrum of NIT-1 (b) The signal strength of the NIT-1 and structure, (c) The mass spectrum of NIT-1.

**Fig. S3** The <sup>1</sup>H NMR test results of NIT-1.

**Fig. S4** The <sup>1</sup>H NMR test results of compound 3.

**Fig. S5** The mass spectrum of NIT-2. (a) The total ion current spectrum of NIT-1 (b) The signal strength of the NIT-2, (c) The mass spectrum of NIT-2.

**Fig. S6** The <sup>1</sup>H NMR test results of NIT-2.

**Fig. S7** Screening for successful pairs of coating antigen/serum. Criterion of success is  $\geq 50\%$  inhibition at 20 ng/mL NIT.

**Fig. S8** Five strains of antibodies were deficient in ic-ELISA standards for two pesticides when coated with different antigens. (a) and (c) The coated antigen was homologous. (b) and (d) The coated antigen was heterologous.

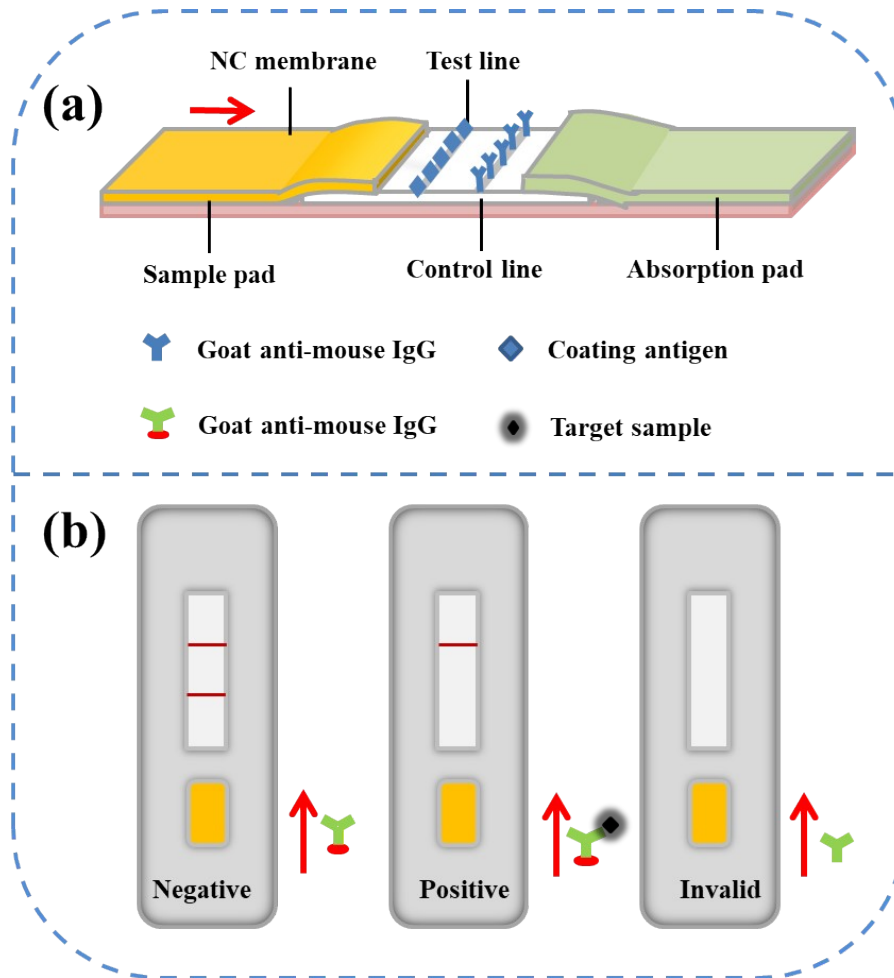
**Fig. S9** Optimize gold-labeled antibody resuspension.

**Table S1.** The titer of mAbs standard curve.

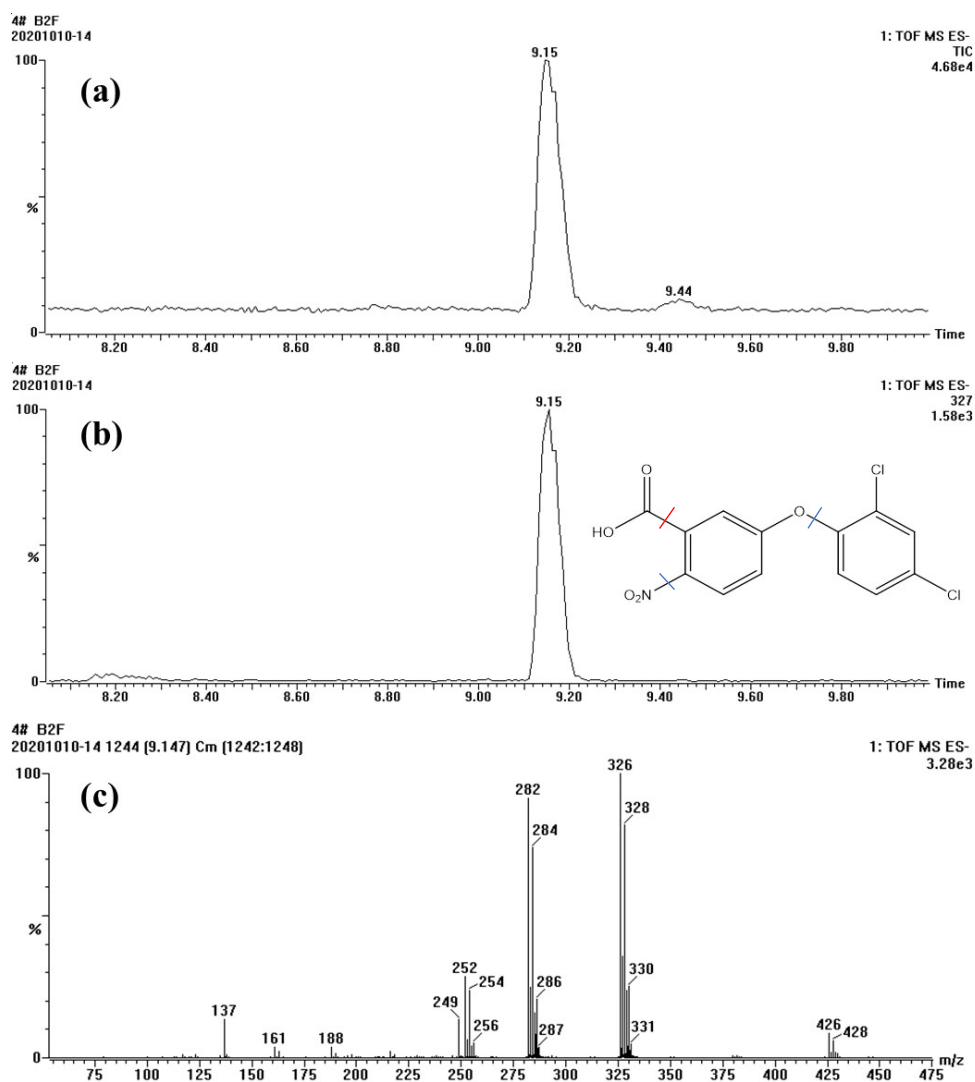
**Table S2.** The IC<sub>50</sub> values of mAbs.

**Table S3.** The affinity constant of mAb 5G7.

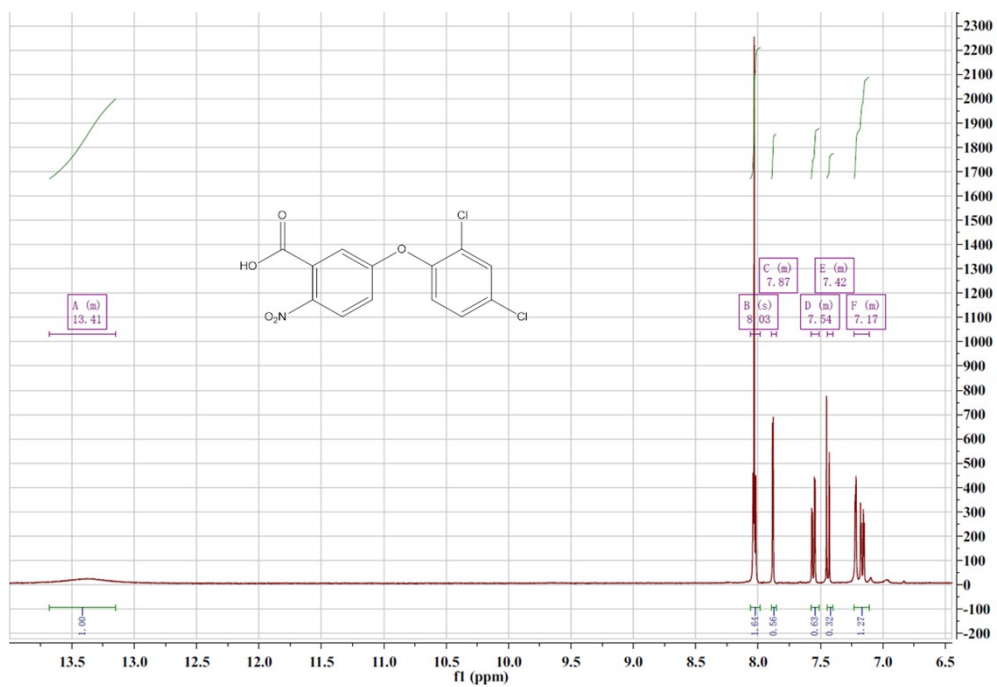
**Table S4.** Cross-reactivity of mAb with NIT and analogues.



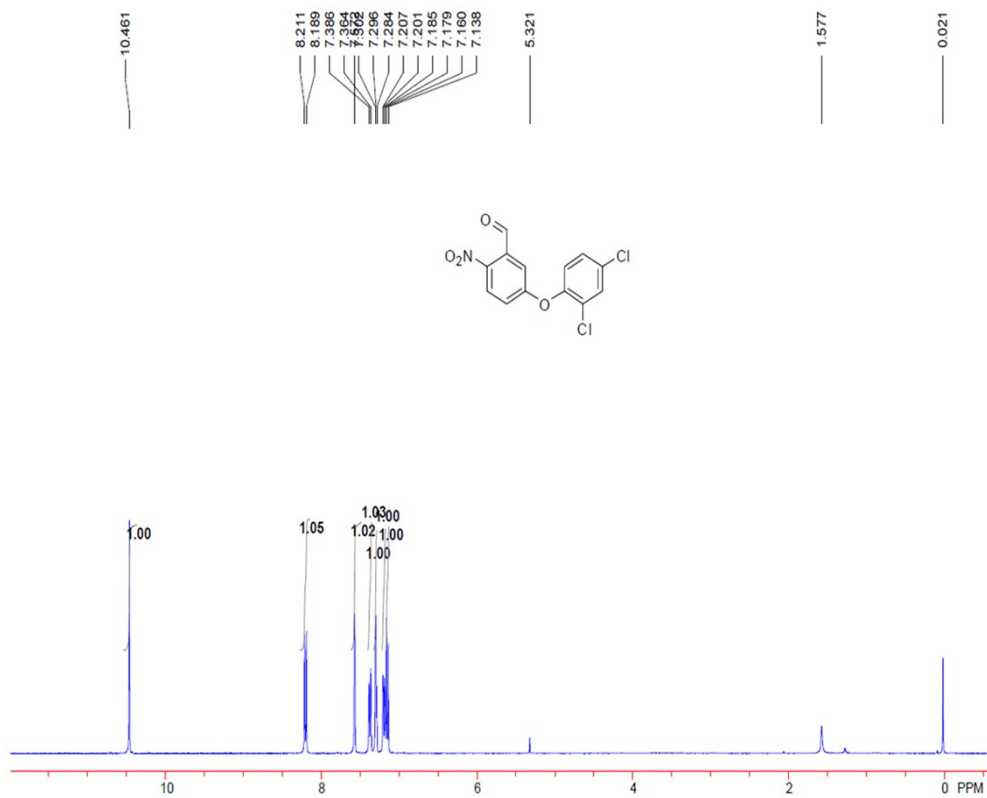
**Fig. S1** Composition of the test strip and the schematic for samples detection.



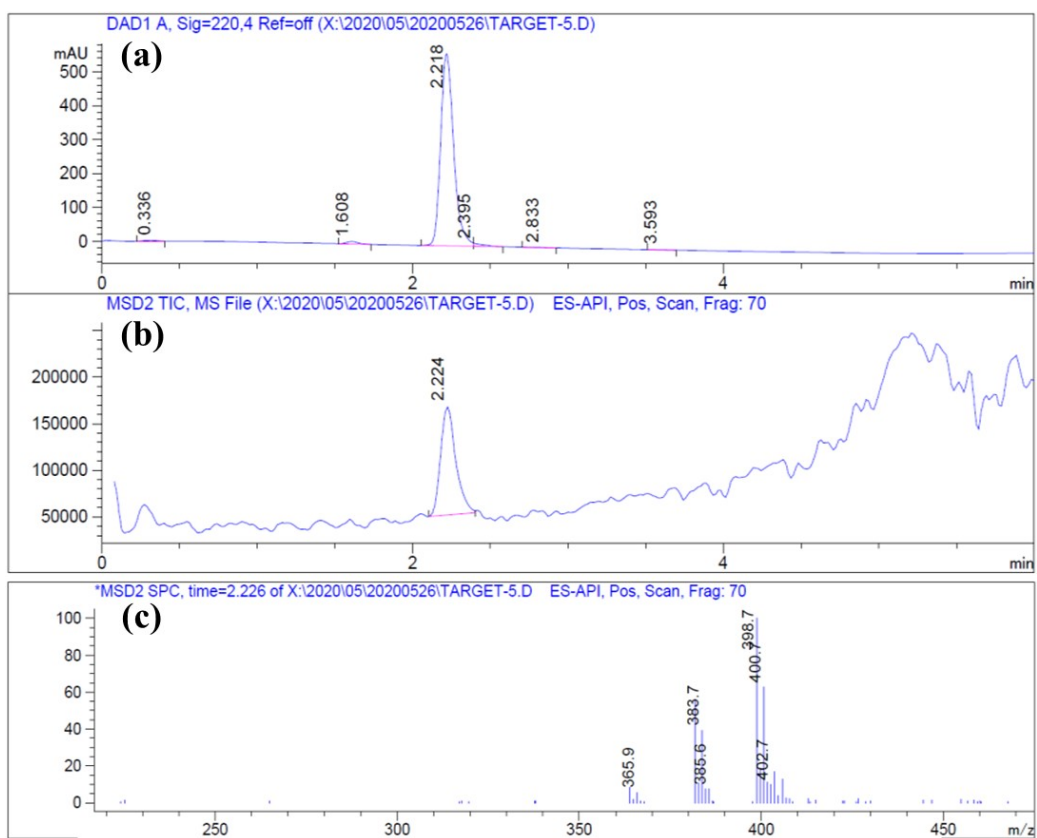
**Fig. S2** The mass spectrum of NIT-1. (a) The total ion current spectrum of NIT-1 (b) The signal strength of the NIT-1 and structure, (c) The mass spectrum of NIT-1.



**Fig. S3** The  $^1\text{H}$  NMR test results of NIT-1.



**Fig. S4** The  $^1\text{H}$  NMR test results of compound 3.



**Fig. S5** The mass spectrum of NIT-2. (a) The total ion current spectrum of NIT-1 (b) The signal strength of the NIT-2, (c) The mass spectrum of NIT-2.

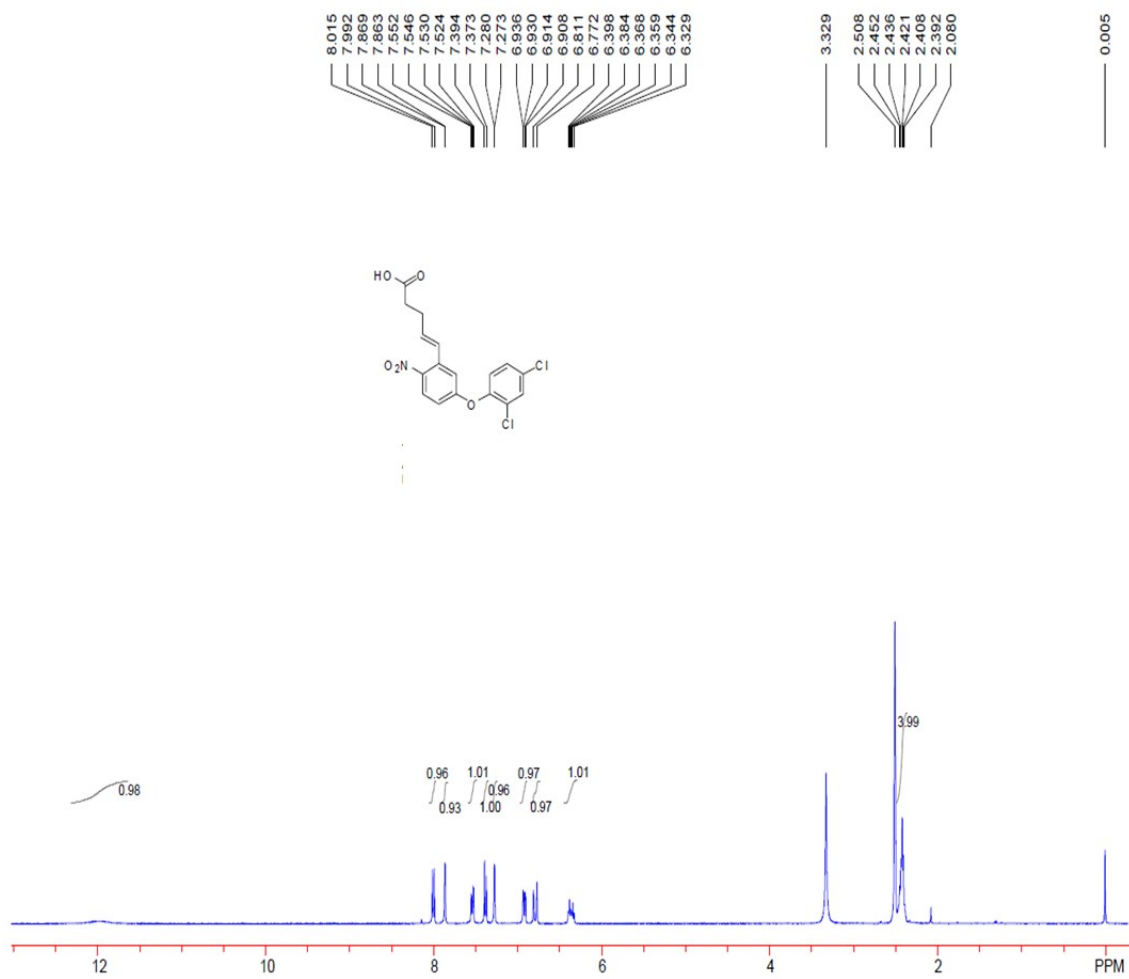


Fig. S6 The <sup>1</sup>H NMR test results of NIT-2

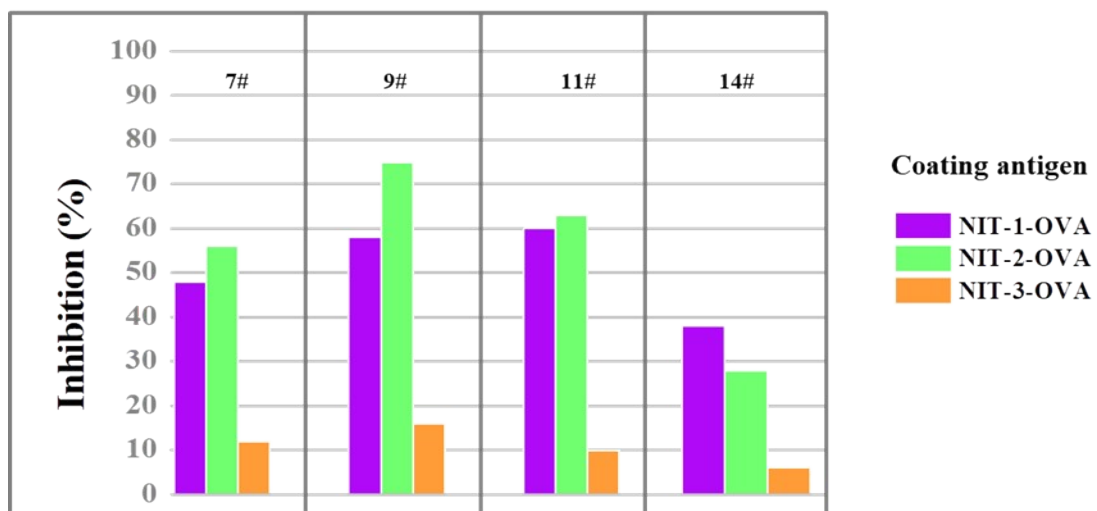
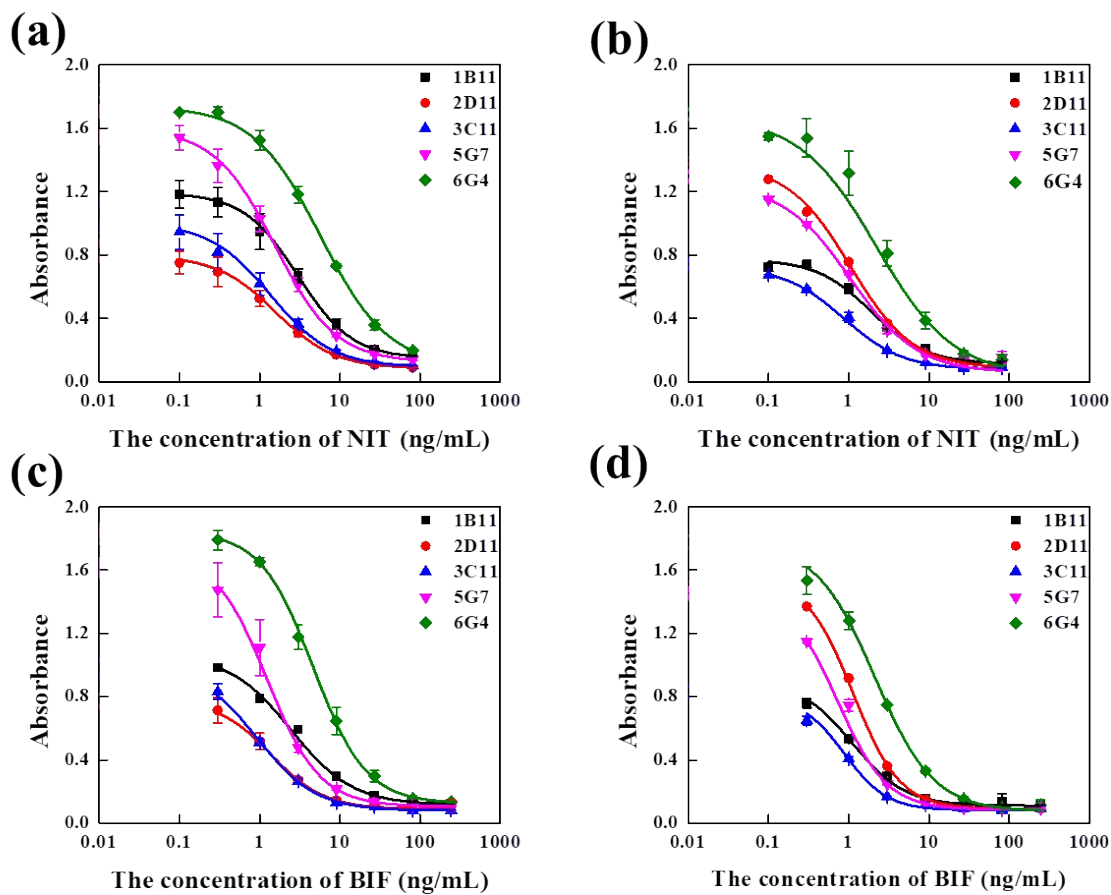
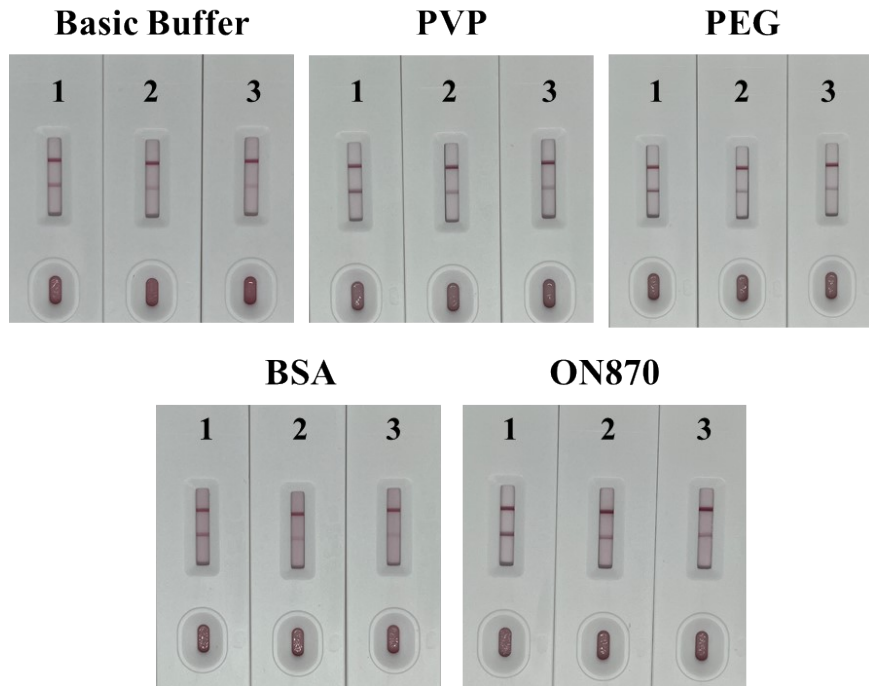


Fig. S7 Screening for successful pairs of coating antigen/serum. Criterion of success is  $\geq 50\%$  inhibition at 20 ng/mL NIT.



**Fig. S8** Five strains of antibodies were deficient in ic-ELISA standards for two pesticides when coated with different antigens. (a) and (c) The coated antigen was homologous. (b) and (d) The coated antigen was heterologous.



**Fig. S9** Optimize gold-labeled antibody resuspension.



**Table S1.** The titer of mAbs standard curve.

Coating antigen (0.1mg/mL)	NIT-1-BSA				NIT-1-ABSA				NIT-2-BSA				NIT-2-ABSA				
	2#		3#		7#		9#		11#		14#		17#		18#		
	DF		DF		DF		DF		DF		DF		DF		DF		
	1000	9000	1000	9000	1000	9000	1000	9000	1000	9000	1000	9000	1000	9000	1000	9000	
NIT-1-OVA	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	+++	++	++	+	+	+
NIT-2-OVA	++	+	+	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	+	++	++
NIT-3-OVA	+	-	-	-	+	-	+	-	+	-	+	-	-	-	-	-	-

Annotation : -, absorbance <0.8; +, absorbance 0.8-1.2; ++, absorbance 1.2-2.0; +++, absorbance >2.0; DF is short for dilution fold.

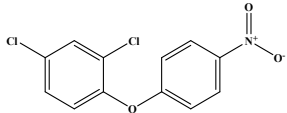
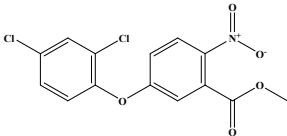
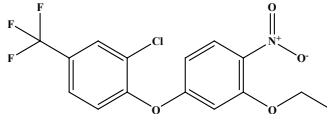
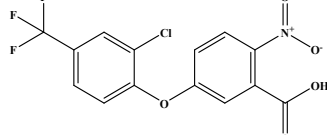
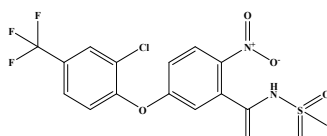
**Table S2.** The IC<sub>50</sub> values of mAbs.

mAbs	Homologous coatings		Heterologous coatings	
	BIF (ng/mL)	NIT (ng/mL)	BIF (ng/mL)	NIT (ng/mL)
1B11	2.98	2.46	1.89	1.10
2D11	1.57	1.38	1.04	1.12
3C11	1.35	0.92	0.86	0.87
5G7	1.54	1.22	1.04	0.73
6G4	5.76	4.64	2.20	2.11

**Table S3.** The affinity constant of mAb 5G7.

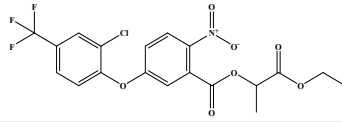
Antigen	Affinity constant
Homologous coatings	2.01×10 <sup>10</sup>
Heterologous coatings	1.54×10 <sup>7</sup>

**Table S4.** Cross-reactivity of mAb with NIT and analogues.

Chemical compound	Structure	IC <sub>50</sub> (ng/mL)	Cross-reaction (%)
Nitrofen		0.87	100
Bifenox		0.86	101
Oxyfluorfen		>5	<11.8
Acifluorfen		>5	<11.8
Fomesafen		>5	<11.8

---

Lactofen



>5

<11.8

---