

## Supporting information

### The method of labeling anti-FIP antibodies with Cy5.5

The anti-FIP antibody was labeled with Cy5.5 according to the manufacturer's instructions. One milliliter of anti-fipronil antibody (0.5 mg/mL) was subjected to dialysis against a NaCl solution (0.15 M) at room temperature. After a duration of 4 hours, the dialysate was substituted with a fresh solution and dialyzed at a temperature of 4 degrees Celsius throughout the night. The solution was replaced with NaHCO<sub>3</sub> (0.1 M), and dialysis continued for 4 hours at RT. The ratio of Cy5.5-NHS to antibody required was calculated to be 20:1. Cy5.5 was dissolved in DMSO to a concentration of 10 mg/mL and slowly added to the antibody solution. The mixture was incubated in a shaker at a low speed at RT for 30 min away from light. The labeled antibodies were harvested following dialysis with 0.15 M NaCl at room temperature and subsequently at 4°C.

Figure S1. Synthesis of coating-antigen FIP-OVA.

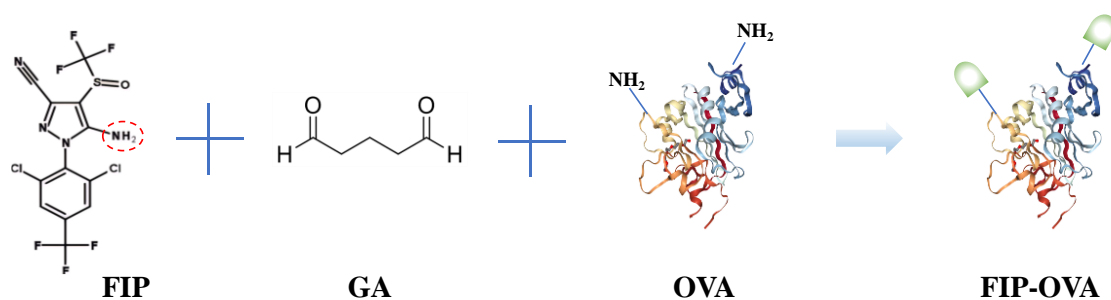


Figure S2. Functionalization of fiber bioprobe using FIP-OVA.

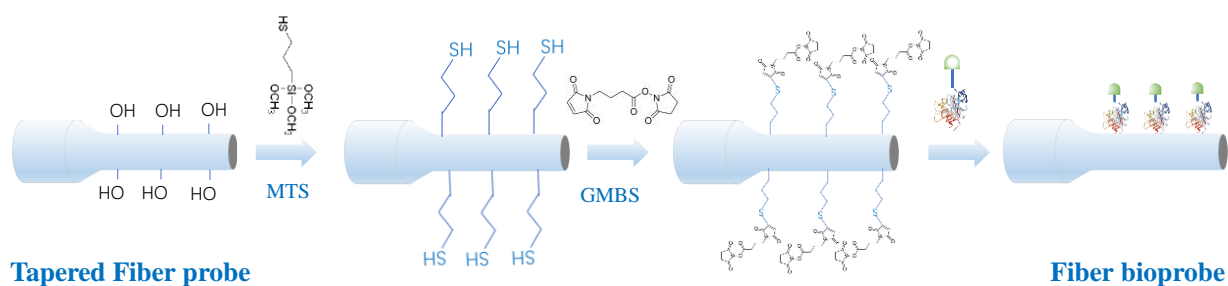


Figure S3. Characteristics of immunogen and coating-antigen. The SDS-PAGE image for the gel electrophoresis testing of FIP-BSA, BSA, FIP-OVA, and OVA. marker: 170, 130, 100, 70, 55, 40, 35, 25, 15, and 10 kDa (Up to down).

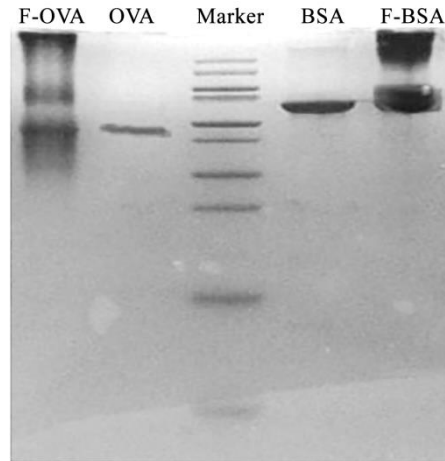
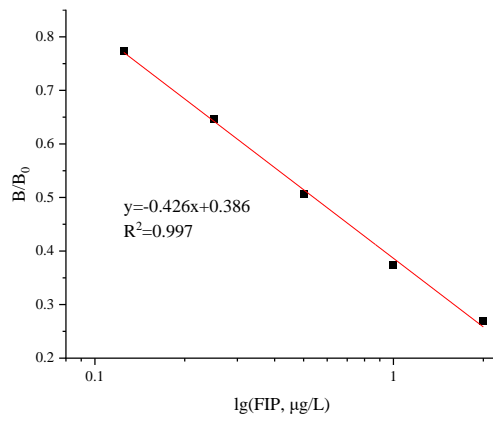
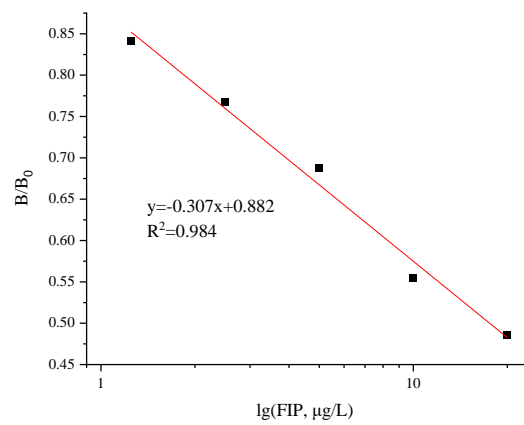


Figure S4. Sensitivity of antibodies. (A) Clone F-3F6. (B) Clone F-4G9



(A)



(B)

Figure S5 Dose-response curve of FIP using ELISA.

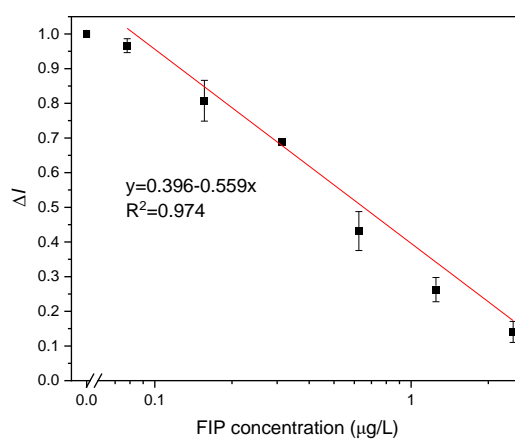


Figure S6 The regeneration performance of the functionalized fiber bioprobe with FIP 0.75  $\mu\text{g/mL}$  antibody.

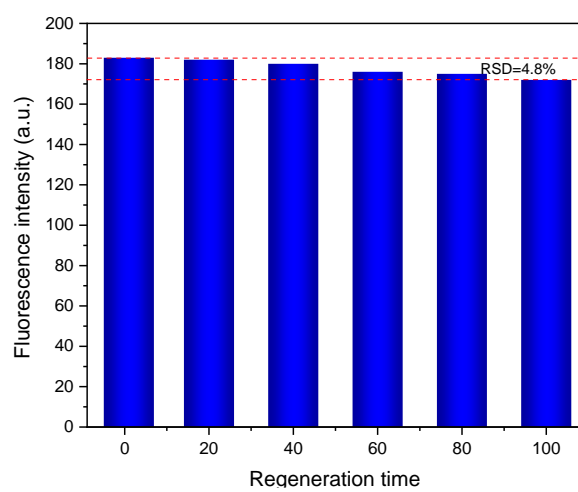


Table S1 Measurement of the concentration and valence of monoclonal antibody

Monoclonal antibody	Concentration (mg/mL)	Valence
F-3F6	8.5	1:2.0×10 <sup>6</sup>
F-4G9	6.7	1:5.12×10 <sup>5</sup>

Table S2 Comparison between other reports and this work on analytical performance of available sensors for simultaneous detection of multiple pesticides

Methods	Quantitation or not	LOD	Detection time	Reusability	Ref
<b>Lateral Flow immunoassay</b>	No	25.0 µg/L	/	No	[1]
<b>Fluorescent immunoassay</b>	1.39 – 53.12 µg/L	210.0 µg/L	45 min	No	[2]
<b>ELISA</b>	0.54 – 12.6 µg/L	0.22 µg/L	150 min	No	[3]
<b>Camel single-domain antibody-based Immunoassays</b>	30.0 – 1000.0 µg/L	10.0 µg/L	150 min	No	[4]
<b>Lateral Flow immunoassay</b>	No	160.0 µg/L	/	No	[5]
<b>icELISA</b>	2.5 – 210.0 µg/L	0.23 µg/L	150 min	No	[6]
<b>Evanescence wave fluorescence biosensor</b>	0.25 – 425.0 µg/L	0.032 µg/L	15 min	Yes	This work

## References:

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2. Sun, X.; Zhang, J.; Lu, Q.; Tian, J.; Kong, D.; Luo, J.; Yang, M. Development of a sensitive fluorescent immunoassay based on fluorescent nanoparticles labeling for the quantitation of fipronil in edible flowers. *LWT* **2023**, *184*, 115113.

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5. Zhou, X.H.; Zhang, C.Q.; Zhang, X.; Sun, C.; Li, J.; Xiao, X.; Ouyang, Q.; Wang, Y. Determination of fipronil and its metabolites in eggs by indirect competitive ELISA and lateral-flow immunochromatographic strip. *Biomed. Environ. Sci.* **2020**, *33*, 731–734.
6. Li, P.; Bai, Y.; Jiang, H.; Zhang, Y.; Li, Y.; Duan, C.; Wen, K.; Yu, X.; Wang, Z. Broad-specificity antibody profiled by hapten prediction and its application in immunoassay for fipronil and major metabolites. *J. Hazard. Mater.* **2023**, *441*, 129931.