Supplementary Material

1. Supplementary Figures and Tables

1.1 Supplementary Figures

Supplementary Figure 1. Optimization of IPTG concentration for 4F5 scFv and 4B9 scFv expression.

(A): The SDS-PAGE of 4F5 scFv, M: marker, 1-5: The concentrations of IPTG were 0, 0.25, 0.5, 0.75, 1.0 mM; (B): the SDS-PAGE of 4B9 scFv, M: marker, 1-5: The concentrations of IPTG were 0, 0.25, 0.5, 0.75, 1.0 mM.
Supplementary Figure 2. Standard curves of the icELISA with 4F5 scFv and 4B9 scFv for the detection of FB$_1$. 
Supplementary Figure 3. The synthesis and purification of FB<sub>1</sub>-FITC and FB<sub>1</sub>–5-DTAF. (A): The synthesis process of FB<sub>1</sub>-FITC and FB<sub>1</sub>–5-DTAF; (B) and (C): Purification of FB<sub>1</sub>-FITC and FB<sub>1</sub>–5-DTAF tracers by thin layer chromatograph.
Supplementary Figure 4. The root mean square deviation (RMSD) values of the scFvs-FBₙ. (A): The RMSD of 4F5-FB₁. (B): The RMSD of 4F5-FB₂. (C): The RMSD of 4B9-FB₁. (D): The RMSD of 4B9-FB₂.
Supplementary Figure 5. The hydrophobicity surface pocket of 4F5-FB₁, 4F5-FB₂, 4B9-FB₁ and 4B9-FB₂. (A): The hydrophobicity surface pocket of 4F5-FB₁. (B): The hydrophobicity surface pocket of 4F5-FB₂. (C): The hydrophobicity surface pocket of 4B9-FB₁. (D): The hydrophobicity surface pocket of 4B9-FB₂.
Supplementary Figure 6. The interpolated charged surface pocket of 4F5-FB1, 4F5-FB2, 4B9-FB1 and 4B9-FB2. (A): The interpolated charged surface pocket of 4F5-FB1. (B): The interpolated charged surface pocket of 4F5-FB2. (C): The interpolated charged surface pocket of 4B9-FB1. (D): The interpolated charged surface pocket of 4B9-FB2.
Supplementary Figure 7. The order of the atoms in the FB\textsubscript{1} and FB\textsubscript{2}. (A): The order of the atoms in the FB\textsubscript{1}; (B): The order of the atoms in the FB\textsubscript{2}.
### 1.2 Supplementary Tables

Table S1. Primers of 4F5 scFv and 4B9 scFv.

| Primer   | Sequence                                                                 |
|----------|---------------------------------------------------------------------------|
| 4F5-VL-F1| GACATTGTGCTCACCCAATCT                                                    |
| 4F5-VL-R1| TTTTATTTCCAGTTTGGTCC                                                     |
| 4F5-VL-F2| CTACACGAGGCCCAGGCCGCGCCGCGCATGAGACATTGTGCTCACCACAACT                    |
| 4F5-VL-R2| GGAGCCGCCGCCAGACCACCAACCACCAACCACCAACCACCAACCACCTTTTT                   |
| 4F5-VH-F1| GAGGTGAAGCTGTTGGAAGTC                                                   |
| 4F5-VH-R1| TGAAGGAGAGCAGTGACTGAGG                                                   |
| 4F5-VH-F2| GGCGGCGGCCACCATGAGATGTGCTTGGAGCTGAAACACT                                 |
| 4F9-VL-F1| GATGTTGAGACCAACACT                                                      |
| 4B9-VL-R1| TTTTATTTCCAGTTTGGTCC                                                     |
| 4B9-VL-F2| CTACACGACAGGCCAGGCCGAGGGATGCTTGGAGCTGAAACACT                             |
| 4B9-VL-R2| GGAGCCGCCGCCAGACCACCAACCACCAACCACCAACCACCAACCACCTTTTT                   |
| 4B9-VH-F1| CAGGTCAACTGCAGCAACC                                                    |
| 4B9-VH-R1| TGAAGGAGACTGACTGAGG                                                     |
| 4B9-VH-F2| GGCGGCGGCCGCTCAGTTGCTGATGCTCAGAACTGACAGCAACC                            |
| 4B9-VH-R2| TCGCTAATCAGTTTGGTGGATCAGTTTGGTGGATCAGTTTGGTGGATCAGTTTGGTGGATCAGTTTGGTGG |
| 4B9-VH-R3| CGGAGTCAGGCCCAGGCGGCTCAGTTTGGTGGATCAGTTTGGTGGATCAGTTTGGTGGATCAGTTTGGTGG  |
| scFv | sequence |
|------|----------|
| **4F5** | GACATTGCTCACCACAACTCCAGCTCTTTTGGCTGTCTCTAGGGCAGAGAGGCCCTCCTCCTGCAGAGCCAGTGAAAGTGTTGAATATTATGGCACAGGTTTAATGCAAGTGGTACCAACAGAAACCAGGACAGCCACCCAAACTCTCTACGTAGATCTGGGGTCCCTGACAGGTTAGTGGCAGTGGGTCTGGGACAGACTTCAACCTCAACATCCATCCTGTGGAGGAGGATGATATTGCAATGTATTTCTGTCAGCAAA |
| **4B9** | GATGTTGTGATGACCCAAACTCCACTCTCCCTGCTGAGCTCTCTTCCTCTCCCTCTCTATGCAGTCTTGGAGATCAGGCCCTCATTGGTACCTGCAGAAGCCAGGCCAGTCTCCAAGCTCCTGATCTACAAAGTTTCCAACCGATTTTCTGGGGTCCCAGACAGGTTCAGTGGCAGTGGATCAGGGACAGATTTCACACTCAAGATCAGCAGAGTGGAGACTGAGGATCTGGGAGTTTATTTCTGCATCAAAGTACACATGTTCCGTACACGTTCGGAGGGGGGACCAAACTGGAAATAAAAA |

Table S2. Sequences of 4F5 scFv and 4B9 scFv.
Table S3. Results of tracer binding with scFv (n=3).

|                | Free tracer | Antibody binding tracer |
|----------------|-------------|-------------------------|
|                | FP (mP)     | SD                      | FP (mP) | SD    | ΔFP (mP) |
| FB₁-FITC       | 50.5        | 1.4                     | 4F5 scFv | 310.9 | 0.7      | 260.4    |
| Rf 0.1         |             |                         | 4B9 scFv | 270.26| 2.9      | 219.76   |
| FB₁-5-DTAF     | 51.6        | 1.3                     | 4F5 scFv | 131.5 | 2.3      | 79.9     |
| Rf 0.1         |             |                         | 4B9 scFv | 142.3 | 1.8      | 90.7     |
| Strategies          | Analytes          | Antibody | Time / steps       | Homogeneous/ Heterogeneous | LOD in (μg/L, μg/kg) | Sample     | Ref   |
|---------------------|-------------------|----------|--------------------|----------------------------|----------------------|------------|-------|
| AuNP-LFIA<sup>a</sup> | FB<sub>1</sub>     | mAb      | 15 min/one step    | Heterogeneous              | 60                   | Corn       | 1     |
| AuNP-LFIA           | FB<sub>1</sub>, FB<sub>2</sub> and FB<sub>3</sub> | mAb      | 15 min/one step    | Heterogeneous              | 11.25 (FB<sub>1</sub>)| Maize      | 2     |
| ICS<sup>b</sup>     | FB<sub>1</sub>     | scFv     | 15 min/one step    | Heterogeneous              | 25 (in buffer)       | Maize      | 3     |
| MB-ELISA<sup>c</sup>| FB<sub>1</sub>     | Mimotope  | 60 min/two steps   | Heterogeneous              | 11.1                 | Maize, wheat | 4     |
| dc-pELISA<sup>d</sup> | FB<sub>1</sub>     | mAb      | 120 min/two steps  | Heterogeneous              | 12.5                 | Corn       | 5     |
| icELISA<sup>e</sup>| FB<sub>1</sub>     | mAb      | 70 min/two steps   | Heterogeneous              | 5.4 (in buffer)      | Maize      | 6     |
| FPIA<sup>f</sup>    | FB<sub>1</sub> and FB<sub>2</sub> | mAb | 10 s/ one step     | Heterogeneous              | 157.4 (FB<sub>1</sub>) and 290.6 (FB<sub>2</sub>) | Maize | 7     |
| FPIA                | FB<sub>1</sub> and FB<sub>2</sub> | scFv     | 10 s/one step      | Heterogeneous              | 441.5 (FB<sub>1</sub>) and 344.9 (FB<sub>2</sub>) | Maize | This study |

<sup>a</sup> Colloidal gold nanoparticle based immunochromatographic test strips.  
<sup>b</sup> Immunochromatographic strip.  
<sup>c</sup> Microarray-based immunoassay.  
<sup>d</sup> Direct competitive plasmonic enzyme-linked immunosorbent assay.  
<sup>e</sup> Indirect competitive enzyme-linked immunosorbent assay.  
<sup>f</sup> Fluorescence polarization immunoassay.
Table S5. Detection of FB₁+FB₂ using FPIA and HPLC-MS/MS in positive maize samples (N=3).

| Samples | FBₐ | HPLC-MS/MS (µg kg⁻¹) | FPIA (µg kg⁻¹) | CV (%) |
|---------|-----|---------------------|----------------|--------|
| 1       | FB₁+FB₂ | 602                | 662            | 8.2    |
| 2       | FB₁+FB₂ | 67                 | -              | -      |
| 3       | FB₁+FB₂ | - a                | - a            | -      |
| 4       | FB₁+FB₂ | - a                | - a            | -      |
| 5       | FB₁+FB₂ | 328                | 415            | 2.4    |
| 6       | FB₁+FB₂ | 1082               | 756            | 8.8    |
| 7       | FB₁+FB₂ | 1668               | 1350           | 4.5    |
| 8       | FB₁+FB₂ | 525                | 782            | 9.1    |
| 9       | FB₁+FB₂ | 10422              | 8964           | 2.9    |

Note: a Not detected.
Table S6. The amino acid composition of 4F5 scFv.

| Amino acid | No. | content | Amino acid | No. | content | Amino acid | No. | content |
|------------|-----|---------|------------|-----|---------|------------|-----|---------|
| Ala (A)    | 12  | 4.8%    | His (H)    | 1   | 0.4%    | Thr (T)    | 16  | 6.4%    |
| Arg (R)    | 13  | 5.2%    | Ile (I)    | 10  | 4.0%    | Trp (W)    | 4   | 1.6%    |
| Asn (N)    | 3   | 1.2%    | Leu (L)    | 16  | 6.4%    | Tyr (Y)    | 14  | 5.6%    |
| Asp (D)    | 11  | 4.4%    | Lys (K)    | 10  | 4.0%    | Val (V)    | 15  | 6.0%    |
| Cys (C)    | 4   | 1.6%    | Met (M)    | 6   | 2.4%    | Pyl (O)    | 0   | 0.0%    |
| Gln (Q)    | 11  | 4.4%    | Phe (F)    | 9   | 3.6%    | Sec (U)    | 0   | 0.0%    |
| Glu (E)    | 11  | 4.4%    | Pro (P)    | 10  | 4.0%    |            |     |         |
| Gly (G)    | 40  | 16.0%   | Ser (S)    | 34  | 13.6%   |            |     |         |
Table S7. The amino acid composition of 4B9 scFv.

| Amino acid | No. | content | Amino acid | No. | content | Amino acid | No. | content |
|------------|-----|---------|------------|-----|---------|------------|-----|---------|
| Ala (A)    | 7   | 2.8%    | His (H)    | 4   | 1.6%    | Thr (T)    | 21  | 8.5%    |
| Arg (R)    | 9   | 3.7%    | Ile (I)    | 6   | 2.4%    | Trp (W)    | 5   | 2.0%    |
| Asn (N)    | 7   | 2.8%    | Leu (L)    | 20  | 8.1%    | Tyr (Y)    | 12  | 4.9%    |
| Asp (D)    | 10  | 4.1%    | Lys (K)    | 13  | 5.3%    | Val (V)    | 15  | 6.1%    |
| Cys (C)    | 4   | 1.6%    | Met (M)    | 4   | 1.6%    | Pyl (O)    | 0   | 0.0%    |
| Gln (Q)    | 13  | 5.3%    | Phe (F)    | 9   | 3.7%    | Sec (U)    | 0   | 0.0%    |
| Glu (E)    | 6   | 2.4%    | Pro (P)    | 10  | 4.1%    |            |     |         |
| Gly (G)    | 37  | 15.0%   | Ser (S)    | 34  | 13.8%   |            |     |         |
Table S8. Detailed information about the predicted interactions between scFvs and FBx.

| Complex | Category | Types | From | To | Distance (Å) |
|---------|----------|-------|------|----|--------------|
| Hydrogen Bond | Conventional Hydrogen Bond | H: ARG108 | FB1: O15 | 1.77 |
| Hydrogen Bond | Conventional Hydrogen Bond | H: ARG108 | FB1: O13 | 1.85 |
| Hydrogen Bond | Conventional Hydrogen Bond | H: THR110 | FB1: O10 | 1.63 |
| Hydrogen Bond | Conventional Hydrogen Bond | H: TYR111 | FB2: H101 | 1.89 |
| Hydrogen Bond | Conventional Hydrogen Bond | H: TYR113 | FB2: O3 | 2.04 |
| 4F5-FB1 | Hydrogen Bond | H: TYR113 | FB2: H103 | 1.70 |
| Hydrogen Bond | Conventional Hydrogen Bond | H: MET115 | FB2: O7 | 2.51 |
| Hydrogen Bond | Conventional Hydrogen Bond | L: TYR55 | FB2: O11 | 1.63 |
| Hydrogen Bond | Conventional Hydrogen Bond | L: GLN105 | FB2: O4 | 1.99 |
| Hydrogen Bond | Conventional Hydrogen Bond | L: GLN105 | FB2: H106 | 1.96 |
| Hydrogen Bond | Conventional Hydrogen Bond | L: TYR42 | FB2: H104 | 2.00 |
| Hydrogen Bond | Conventional Hydrogen Bond | L: ARG108 | FB2: O10 | 1.78 |
| Hydrogen Bond | Conventional Hydrogen Bond | L: TYR31 | FB2: O13 | 1.67 |
| Hydrogen Bond | Conventional Hydrogen Bond | L: TYR34 | FB2: O13 | 1.61 |
| 4F5-FB2 | Electrostatic force | Attractive Charge | L: LYS109 | FB2: O14 | 2.79 |
| Electrostatic force | Attractive Charge | L: ASP1 | FB2: O14 | 5.08 |
| Hydrophobic force | Pi-Alkyl | L: TYR31 | FB2: C25 | 4.34 |
| Hydrogen Bond | Conventional Hydrogen Bond | H: TRP38 | FB2: O12 | 1.72 |
| Hydrogen Bond | Conventional Hydrogen Bond | L: HIS31 | FB2: O5 | 2.43 |
| Hydrogen Bond | Conventional Hydrogen Bond | L: TYR38 | FB2: O6 | 1.70 |
| Hydrogen Bond | Conventional Hydrogen Bond | L: TYR55 | FB2: O9 | 1.74 |
| Hydrogen Bond | Conventional Hydrogen Bond | L: TYR55 | FB2: O13 | 2.74 |
| Hydrogen Bond | Conventional Hydrogen Bond | L: SER105 | FB2: H104 | 1.81 |
| Hydrogen Bond | Conventional Hydrogen Bond | L: SER106 | FB2: H105 | 1.76 |
| Hydrogen Bond | Conventional Hydrogen Bond | L: TYR116 | FB2: H106 | 1.88 |
| Electrostatic force | Salt bridge | H: LYS55 | FB2: O8 | 2.36 |
| 4B9-FB1 | Electrostatic force | Salt bridge | H: LYS55 | FB2: O14 | 1.81 |
| Electrostatic force | Salt bridge | L: LYS56 | FB2: O9 | 1.93 |
| Electrostatic force | Salt bridge | L: LYS56 | FB2: O11 | 2.09 |
| Electrostatic force | Attractive charge | L: ARG108 | FB2: O13 | 4.50 |
| Electrostatic force | Pi-cation | L: PHE118 | FB2: N16 | 4.29 |
| Hydrophobic force | Pi-Alkyl | L: HIS31 | FB2: C27 | 4.94 |
| Hydrophobic force | Pi-Alkyl | L: HIS31 | FB2: C25 | 4.44 |
| Hydrophobic force | Pi-Alkyl | L: TYR38 | FB2: C25 | 4.28 |
| Hydrogen Bond | Conventional Hydrogen Bond | H: ARG108 | FB2: H103 | 2.10 |
| Hydrogen Bond | Conventional Hydrogen Bond | H: ARG108 | FB2: H104 | 1.96 |
| Hydrogen Bond | Conventional Hydrogen Bond | L: TYR55 | FB2: H101 | 3.01 |
| Hydrogen Bond | Conventional Hydrogen Bond | L: TYR55 | FB2: O10 | 2.72 |
| 4B9-FB2 | Hydrogen Bond | L: TYR55 | FB2: O12 | 1.72 |
| Hydrogen Bond | Conventional Hydrogen Bond | L: TYR56 | FB2: O12 | 1.81 |
| Hydrogen Bond | Conventional Hydrogen Bond | L: TYR38 | FB2: O14 | 1.69 |
| Hydrogen Bond | Conventional Hydrogen Bond | H: TYR38 | FB2: O11 | 1.80 |
| Hydrogen Bond | Conventional Hydrogen Bond   | H: LYS55 | FB2: O7 | 2.26 |
|--------------|-------------------------------|---------|--------|------|
| Electrostatic| Salt bridge                   | L: LYS56| FB2: O14| 2.86 |
| Electrostatic| Salt bridge                   | H: LYS55| FB2: O9 | 1.88 |
| Electrostatic| Salt bridge                   | H: LYS55| FB2: O13| 1.74 |
| Electrostatic| Pi-Alkyl                      | L: HIS31| FB2: N15| 4.64 |

*a* is a bond that Protein-donating OH or NH group approaches the lone pair of an O or N atom. *b* is between two oppositely charged objects. *c* is interaction of pi-electron cloud over an aromatic group and electron group of any alkyl group. *d* is a combination of two non-covalent interactions: hydrogen bonding and ionic bonding. *e* is the interaction between an electron deficient (π-acidic) aromatic system and an anion.
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