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Electronic Supplementary Information

Monoclonal antibody-based immunosensor for the electrochemical detection of Imidacloprid pesticide

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HPLC/MS-MS method

HPLC-MSMS experiments for the determination of IMD were carried out using a liquid chromatograph (Agilent 1260 Infinity) coupled to a triple quadrupole mass spectrometer (Agilent 6460). The column used was Agilent Poroshell 120 EC-C18 2.7 µm, 3x50 mm, with a temperature of 40°C. The binary and isocratic pump flows were 0.8 mL min^{-1} (water) and 0.450 mL min^{-1}, respectively. The mobile phases were (A) water/methanol (80:20) with 5 mM ammonium acetate and (B) acetonitrile with 5 mM ammonium acetate.

The conditions of mass spectrometry were the following: Gas temperature: 330°C; Drying gas flow: 11 L min^{-1}; Nebulizer pressure: 40 psi; Capillary voltage: 4000V. A precursor ion of 256.0596 Unit/Enh and two ions product of 209.0589 and 175.069 Unit/Enh are obtained for IMD.

ELISA kit method

The ELISA test procedure is as follows:

1. Add 50 µL of assay buffer solution to the individual wells successively using a multi-channel pipette or a stepping pipette.
2. Add 50 µL of the standard solutions and samples or sample extracts into the wells of the test strips. We recommend using duplicates or triplicates.
3. Add 50 µL of enzyme conjugate solution to the individual wells successively using a multi-channel pipette or a stepping pipette.
4. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circulation motion on the benchtop for 30 seconds. Be careful not to spill contents.
5. Incubate the strips for 60 minutes at room temperature.
6. After incubation, remove the covering and vigorously shake the contents of these wells into a sink. Wash the strips three times using the 1X washing buffer solution. Use 250 µL of washing buffer for each well and each washing step. Remaining buffer in the wells should be removed by patting the plate dry on a stack of paper towels.
7. Add 150 µL of substrate (color) solution to the wells. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for 30 seconds. Incubate the strips for 20-25 minutes at room temperature. Protect the strips from direct sunlight.
8. Add 100 µL of stop solution to the wells in the same sequence as for the substrate solution.
9. Read the absorbance at 450 nm using a microplate ELISA photometer within 15 minutes after the addition of the stopping solution.

Control assays

Control assays were performed as detailed in the main text. The results are shown in Figure S1.

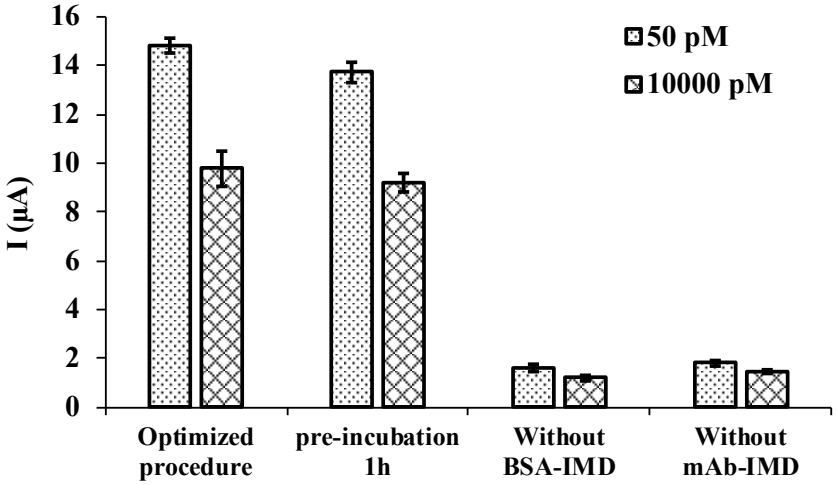

Figure S1. Control assays performed for 2 different IMD concentrations (50 pM and 10000 pM) under the following conditions: i) optimized procedure; ii) with an antibody-IMD pre-incubation of 1h; iii) without immobilizing BSA-IMD on the electrode; iv) without adding the anti-IMD antibody.

