Supporting Information

# A Magnetic-Bead-Based Immunoassay with a Newly Developed Monoclonal Antibody for Rapid and Highly Sensitive Detection of Forchlorfenuron

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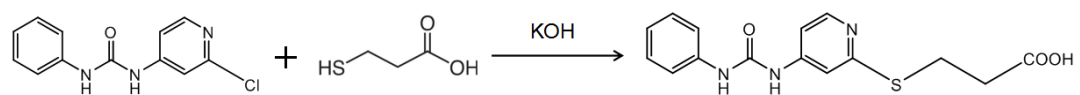
**Figure S1.** Synthesis route of hapten CPPU-COOH. CPPU was coupled with 3-mercaptopropionic acid, and an alkyl mercaptoacid was introduced on the pyridine ring of CPPU.
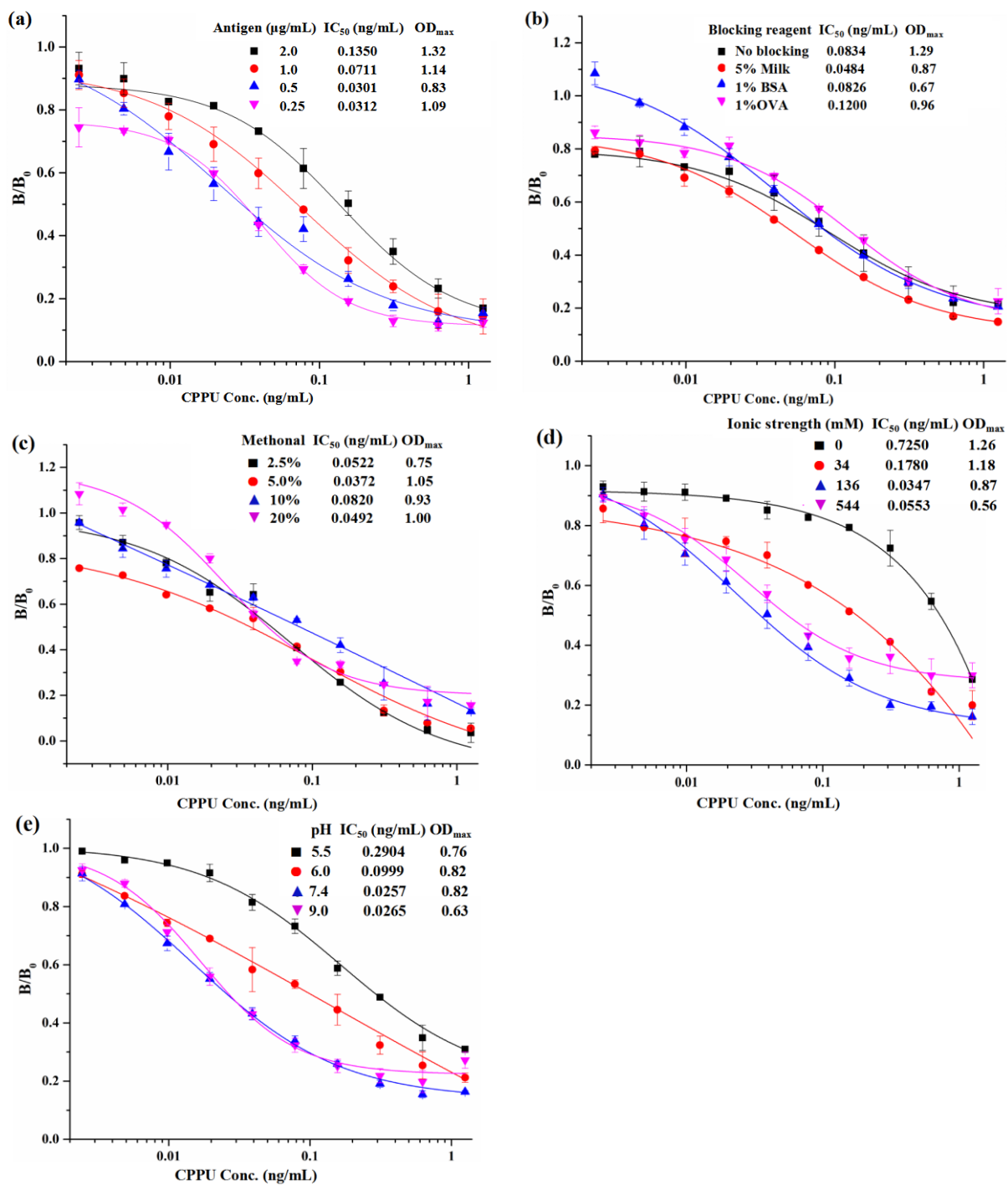
**Figure S2.** Optimization of the operating conditions of icELISA. (a) coating antigen and mAb 14G1 concentration, (b) blocking reagent, (c) methanol concentration, (d) ionic strength in PBS buffer, and (e) pH of buffer.
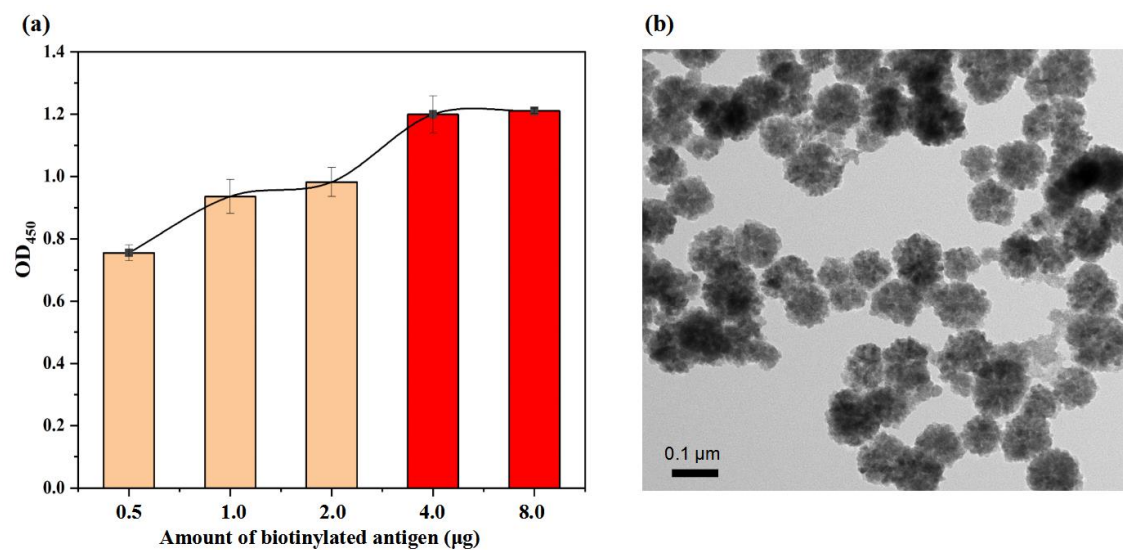
**Figure S3.** (a) Optimization of the amounts of biotinylated CPPU-BSA conjugated to the streptavidin-modified magnetic beads, and (b) characterization of MB-CPPU-BSA conjugate by transmission electron microscope (TEM) image.
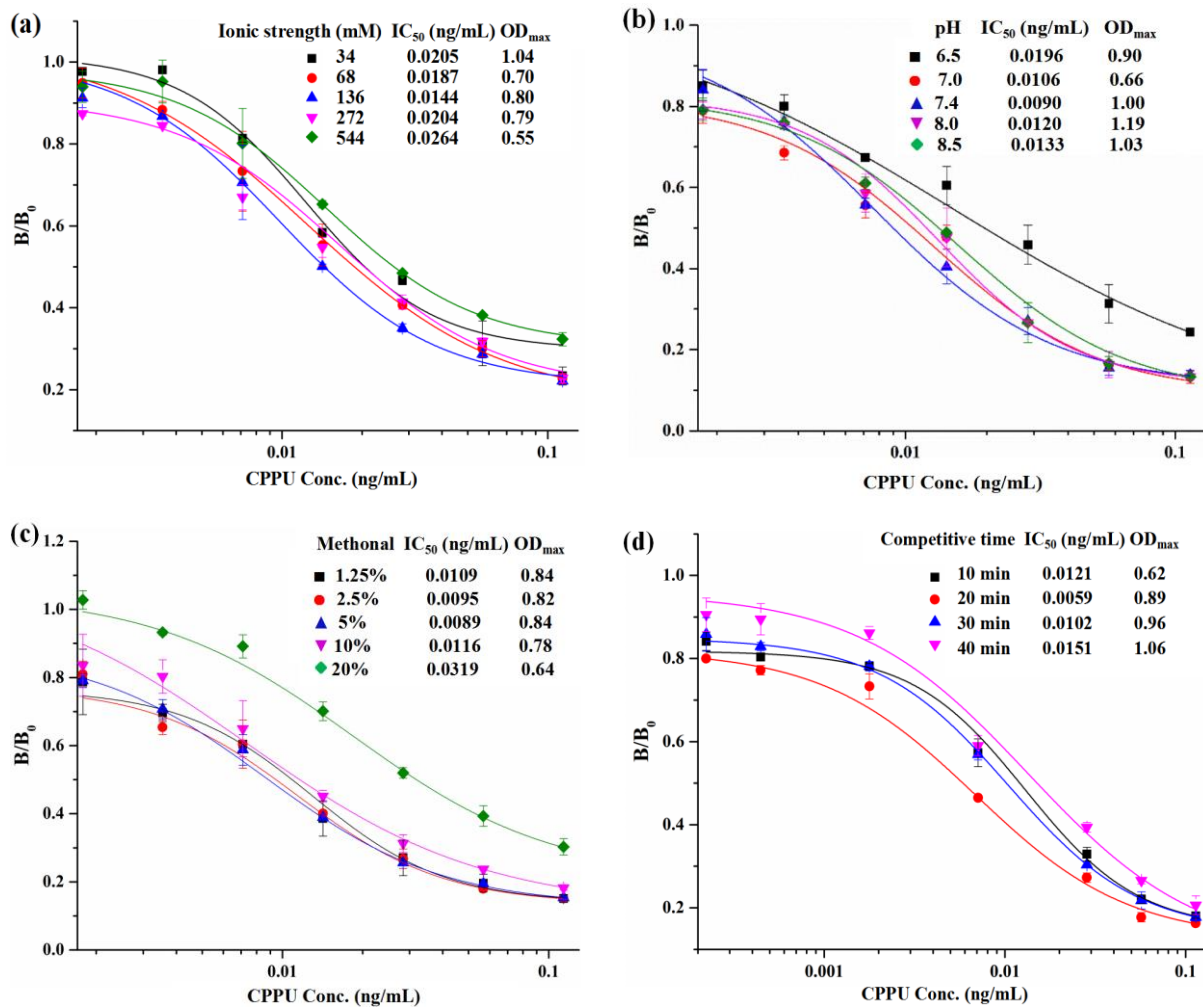
**Figure S4.** Optimization of the operating conditions of MB-based assay. (a) ionic strength in PBS buffer, (b) pH of buffer, (c) methanol concentration, and (d) competitive reaction time.

**Table S1.** Optimization of concentrations of mAb-HRP and MB-CPPU-BSA for MB-based assay.

| Dilution factor of mAb-HRP | MB-CPPU-BSA concentration (μg/μL) | $IC_{50}$ (ng/mL) | $OD_{max}$ |
|---|---|---|---|
| 1:400 | 0.0125 | 0.0315 | 0.921 |
| 1:800 | 0.025 | 0.0147 | 0.863 |
| 1:1600 | 0.05 | 0.0139 | 0.812 |
| 1:3200 | 0.10 | **0.0132** | 0.921 |
| 1:6400 | 0.20 | 0.0140 | 0.833 |

**Table S2.** Analytical performance of the reported immunoassays for CPPU detection.

| Method | Recognition reagent | $IC_{50}$ (ng/mL) | LOD (ng/mL) | Linear range (ng/mL) | Assay time | Reference |
|---|---|---|---|---|---|---|
| dcELISA | pAb | 0.077 | 0.012 | 0.025-0.276 | 70 min | [13] |
| dcELISA | mAb | 0.063 | 0.01 | -[a] | 70 min | [14] |
| LFIA[b] | mAb | 0.286 | 0.045 | 0.089-0.916 | 30 min | [15] |
| icELISA | mAb | 0.05 | 0.013 | 0.022-0.156 | 130 min | [15] |
| icELISA | mAb | 1.04 | 0.16 | 0.31-3.43 | 80 min | [16] |
| MB-based assay | mAb | 0.0061 | 0.0004 | 0.0016-0.0259 | 35 min | This work |
| icELISA | mAb | 0.0253 | 0.002 | 0.005-0.231 | 135 min | This work |

Notes: [a] Data not provided; [b] LFIA means lateral flow immunoassay.

# References

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