Supplementary Information

Buffers and Solutions

The monoclonal antibodies (McAbs)[1] and hapten of triazophos (THHe and THBu) were made from Zhejiang University. Briefly, BALB/c female mice (8-10 weeks of age) were immunized subcutaneously with BSA-THHe conjugate (100 μg) in phosphate-buffered saline (PBS) and complete Fround's adjuvant 1:1 (v/v) initially and subsequently using incomplete Fround's adjuvant at 2 week intervals three times. One week after the last injection, mice were tail bled to check for antibody activity. Once this was found to be positive, the mouse was given one more booster injection with 100 μg of conjugate in PBS (without adjuvant), it was killed 3 days later, its spleen was removed, and the spleen cells were fused with SP2/0 murine myeloma cells. Cell fusion procedures were carried out essentially. Culture supernatants were screened for the presence of antibodies that recognized triazophos 12-14 days after cell fusion. The screening consisted of the simultaneous performance of noncompetitive and competitive indirect ELISAs to test the ability of antibodies to bind the hapten-OVA conjugate and to recognize triazophos. Selected hybridomas were cloned by limiting dilution using HT media on a feeder layer of BALB/c thymocytes (~106 cells/well) and peritoneal macrophages (~5000 cells/well). Stable antibody-producing clones were expanded and cryopreserved in liquid nitrogen. After titer testing with indirect ELISA of the mice ascites, the McAbs were separated by the method of salting out (with caprylic acid-ammonium sulfate) and then were purified by an Immuno-Pure (A) IgG purification kit (Pierce, USA). Indirect ELISA format was used to characterize for reactivity of the McAbs to hapten conjugates. Results

showed that mice ascites displayed a high level for each relative homologous and heterologous hapten (THHe and THBu) conjugates with favorable titers (1.28×106-1.02×107). Several other organophosphorus insecticides or analogues (chlorpyrifos, diazinon, malathion, parathion, methyl parathion and fenitrothion) were tested for cross-reactivities (CRs) to evaluate the specificity of the McAb. The CR for diazinon was 0.03%, and the CRs for the other organophosphorus pesticide were all below 0.01%. The McAb stock solution was prepared as follows: 3.9 mg/mL McAb was separated into 250 μL of solution, which was placed in 0.5 mL centrifugal tubes and stored at -18ºC for future use.

Phosphate-buffered saline (PBS) (pH 7.4, 0.01 mol/L) was prepared as follows: 0.27 g of KH_2PO_4, 2.86 g of $Na_2HPO_4 \cdot 12H_2O$, 0.2 g of KCl, and 8.8 g of NaCl were weighed and dissolved into water and the pH value was adjusted to 7.4. Then, distilled water was added to a constant volume of 1000 mL, and the buffer was stored at 4ºC.

MES buffer (pH 6.0, 15 mmol/L) was produced as follows: 0.32 g of MES (molecular mass 213.25) was dissolved into 90 mL of deionized water, and then the pH value was adjusted to 6.0. Then, distilled water was added to a constant volume of 100 mL, and the buffer was stored at 4ºC.

10 mmol/L EDC buffer was prepared by dissolving 10 mg of EDC (molecular mass 191.7) into 1 mL of deionized water prior to use.

10 mmol/L NHS buffer was made by dissolving a certain amount of NHS into deionized water before use.

1% PBST solution was obtained by adding 1% Tween to phosphate buffer (pH 7.4, 0.01 mol/L). Blocking solution was prepared by dissolving 2% BSA in phosphate buffer (pH 7.4, 0.01 mol/L).

Synthesis of Carboxyl-functioned Fe3O4 Magnetic Nanoparticles(CMNPs)

The carboxyl-functionalized magnetic Fe_3O_4 nanoparticles (CMNPs) were prepared according to the method described in the literature with moderate modification[2].

Hydrophobic magnetic nanoparticles coated with oleic acid were prepared from ferric chloride and ferrous chloride by co-precipitation, with oleic acid used as a surfactant. 8.1 g of $FeCl_3 \cdot 6H_2O$ and 3.3 g of $FeCl_2 \cdot 4H_2O$ were obtained and made into a 150 mL of solution before being moved to a triple-neck flask and heated to 70ºC under the protection of N_2 with vigorous stirring. 18 mL of ammonia solution (25%, w/v) was quickly added under intense stirring. Under vigorous stirring, 4.66 g of oleic acid ($C_{18}H_{34}O_2$), the structure of which is shown in Fig. 1-A, was added to the Fe_3O_4 solution. Under the protection of N_2, the solution was constantly stirred at 70ºC for one hour so that the oleic acid wrapped the nanoparticles. After the reaction, a black precipitate were obtained. The precipitates were separated from the reaction system under external magnetic fields. Excess oleic acid was washed by ethyl alcohol two times and by water three times to remove NH_4Cl and extra oleic acid. Then, 160 mL of a 10 mg/mL $KMnO_4$ solution was used to synthesize azelaic acid ($HOOC(CH_2)_7COOH$) (Fig. 1-A) to obtain carboxylated magnetic nanoparticles. The -C=C- bond of oleic acid coated on the surface of particles was oxidized into -COOH

by $KMnO_4$ solution. The precipitates (Fig. 1-B) were separated by a magnet after 8 h sonication and washed three times with water to remove extra $KMnO_4$. The ferrofluid was obtained by adding water to the precipitates. The ionic equation for the reaction is as follows:

$$Fe^{2+} + 2Fe^{3+} + 8OH^- = Fe_3O_4\downarrow + 4H_2O \qquad (1)$$

Preparation of Immunomagnetic beads(IMBs)

The CMNPs were functionalized with antibodies by cross-linking carboxyl groups on the surface of the CMNPs with amine groups in the antibodies. 1 mL of CMNPs were first obtained and placed into a centrifuge tube, and then 1 mL of MES solution was added. The mixture was rotated for 10 seconds. Then, 1 mL of activation buffer was used to rewash the magnetic beads twice, and 500 μL of MES solution was added. After that, 500 μL of carbodiimide and 500 μL of N-hydroxysuccinimide were separately added and mixed for 30 min with slow rotation at room temperature. Subsequently, the CMNPs were washed three times with PBST, and then 800 μg of McAb was added and incubated for 16–18 h at 37ºC with slight stirring. Afterwards, the IMBs were washed twice with PBST to remove excess McAbs by a magnetic separation process. The non-specific sites on the IMBs were blocked by incubating with PBS buffer (containing 2% BSA) at room temperature for 30 min with slight stirring. Finally, the IMB probes were obtained and stored at 4ºC for further use

Preparation of Hapten-HRP(Horseradish peroxidase) conjugates

The hapten-HRP conjugates were synthesized as described earlier[3]. Horseradish peroxidase (HRP) was conjugated with hapten at a ratio of 1:20. HRP solution (10

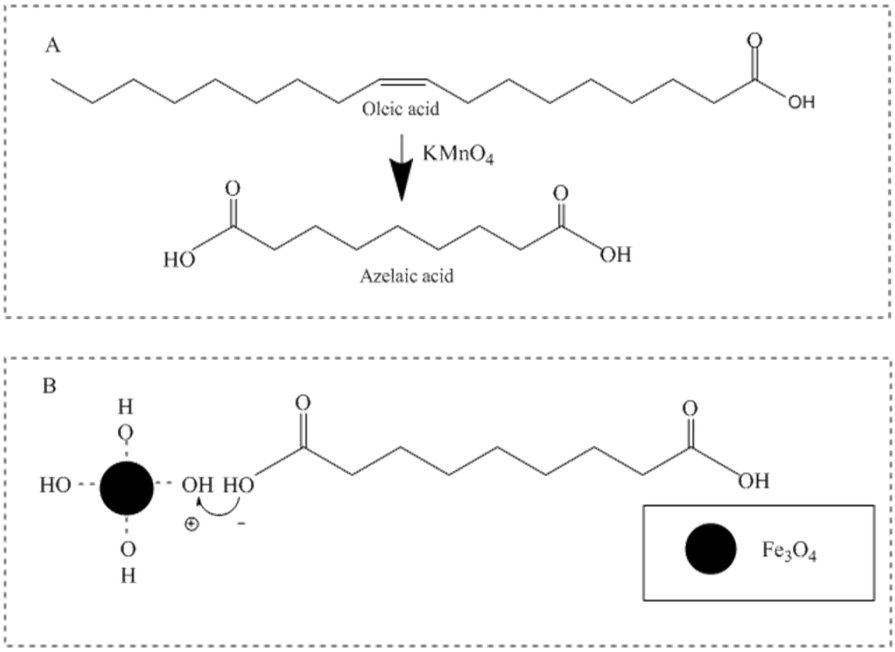
mg/mL) was prepared in carbonate buffer solution for hapten conjugates. 11.1 of mg Hapten was dissolved into 1 mL of DMF solution, and then 0.6 mmol of NHS was added and stirred for 15 min for reaction. After that, 0.3 mmol of DCC was added to the solution, which was stirred at room temperature and kept overnight. Then, the mixture was centrifuged for 5 min at 10,000 rpm to remove the urea precipitate. 300 μL supernatant solution was used to prepare the conjugates with HRP. After the reaction, the solution was placed into a dialysis bag and dialyzed against distilled water three times. In the following 3 days, PBS (0.01 mol/L) was used to dialyze the solution with the refreshed dialysate 3-4 times per day. Finally, glycerin of the same volume was added, evenly mixed, and separately stored at 20ºC.

References

1. J. G. Wen, R. Y. Jin, Z. L. Chen, J. L. Cheng, G. N. Zhu, Anal. Biochem., 2006, 357, 9-14.

2. G. Chen, Y. H. Ma, P. F. Su and B. S. Fang, Biochem. Eng. J., 2012, 67, 120-125.

3. C. Z. Liang, W. J. Gui and G. N. Zhu, J. Chin. Inst. Food Sci. Technol., 2008, 8(6), 102-108.



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108 Fig. 1 Synthesis mechanism of carboxyl- functionalized Fe_3O_4 nanoparticles

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