

Supplementary Information

Caption:

1. Characterization of B[a]P antigens.
2. The antisera of immunized mice detected by *ic*ELISA.
3. Identification of anti-B[a]P mAb.
4. The cross-reactivities of anti-B[a]P mAb detected by *ic*ELISA.
5. Sample preparation detected by HPLC-FLD.
6. The optimization of the GNP- ICS assay.
7. Matrix effect on test strip.
8. The GNP- ICS assay for detection of B[a]P in tea-seed oil.

1. Characterization of B[a]P antigens

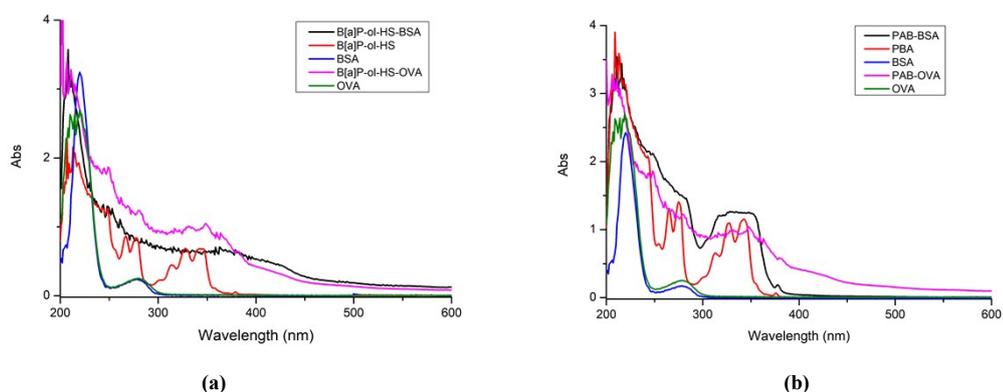


Figure S1. The UV-Vis spectrophotogram of B[a]P haptens and antigens. (a) The UV-Vis spectrophotogram of B[a]P-ol-HS-BSA/OVA; (b) The UV-Vis spectrophotogram of PBA-BSA/OVA.

2. The antisera of immunized mice detected by *icELISA*.

Table S1 The antisera of immunized mice detected by *icELISA*.

Coating antigen	IC ₅₀ (ng mL ⁻¹)	
	Immunized by B[a]P-ol-HS - BSA	Immunized by PBA-BSA
B[a]P-ol-HS-OVA	92.7	62.7
PBA-OVA	35.6	105.6

3. Identification of anti-B[a]P mAb

3.1 The purification of anti-B[a]P mAb

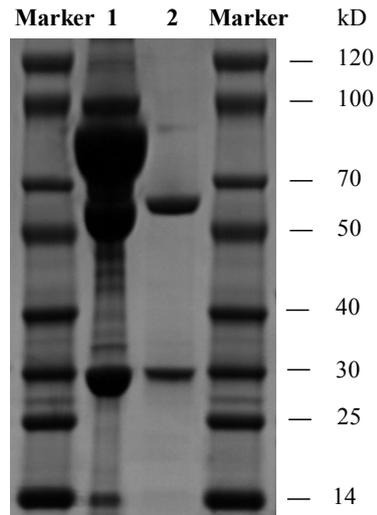


Figure S2. The SDS-PAGE of anti-B[a]P mAb purified by CA-SA. 1, 2 represent the unpurified and purified anti-B[a]P mAb, respectively.

3.2 The subtype of anti-B[a]P mAb

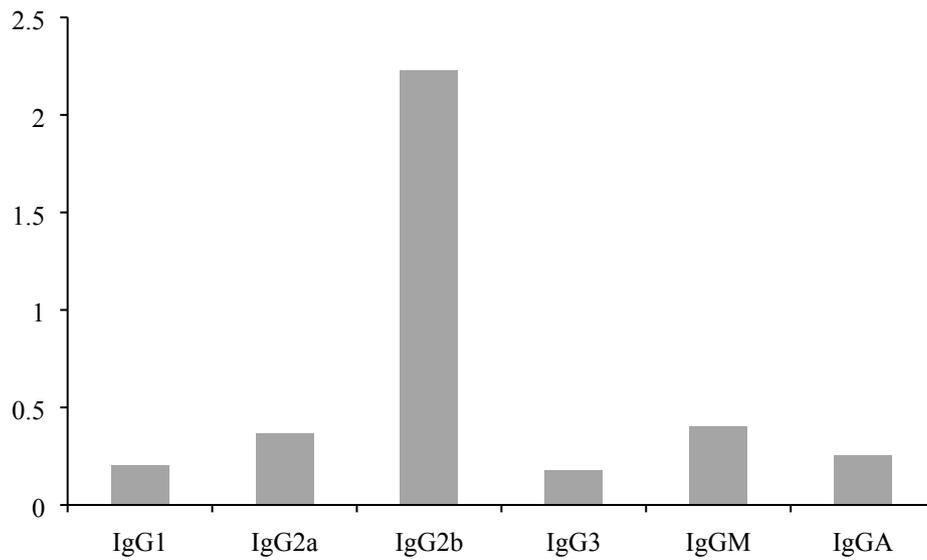


Figure S3. The subtype of anti-B[a]P mAb detected by ELISA.

3.2 Titer of purified anti-B[a]P mAb

Table S2. Titer of purified anti-B[a]P mAb detected by ELISA.

Dilution of purified anti-B[a]P mAb										
	3×10^3	6×10^3	1.2×10^4	2.4×10^4	4.8×10^4	9.6×10^4	1.92×10^5	3.84×10^5	7.68×10^5	Blank
OD ₄₅₀	2.76	2.56	2.41	2.35	2.06	1.72	1.43	0.65	0.27	0.032

As shown in Table S2, the titer of purified anti-B[a]P mAb was up to 7.68×10^5 .

3.3 Affinity curve for anti-B[a]P mAb

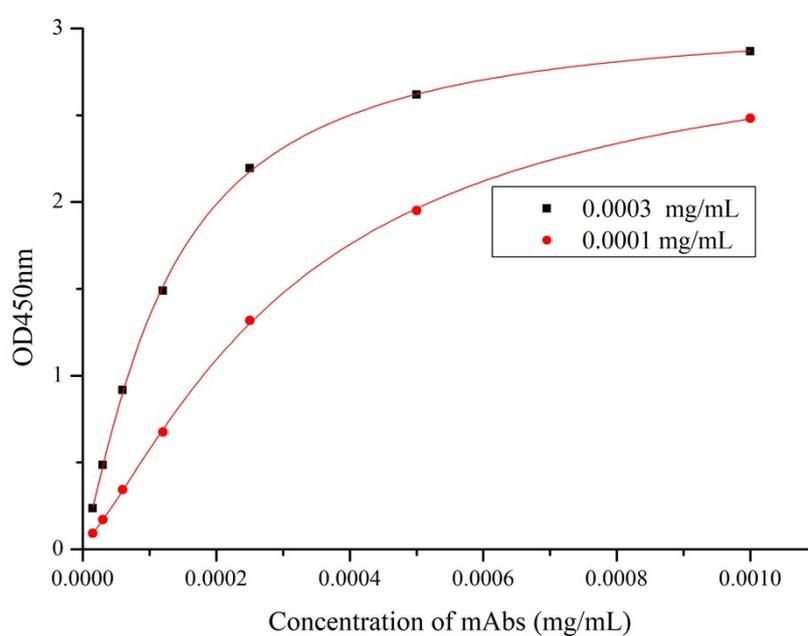
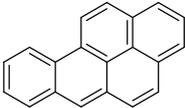
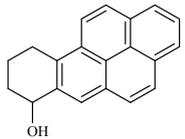
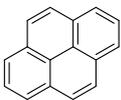
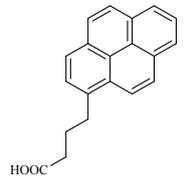
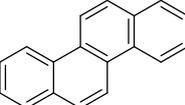
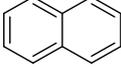
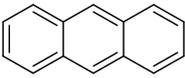
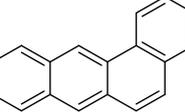
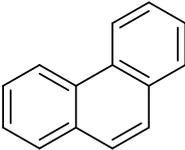


Figure S4. Affinity curve for anti-B[a]P mAb.

The average affinity constant was calculated to be $5.36 \times 10^8 \text{ L mol}^{-1}$.

4. The cross-reactivities of anti-B[a]P mAb detected by *ic*ELISA

Table S3. The cross-reactivity of anti- B[a]P mAbs with PAH analogues determined by *icELISA*.

B[a]P and its analogues	Molecular structure	IC ₅₀ (μg/L)	CR (%)
B[a]P		2.51	100
B[a]P -7-ol		1.25	200.87
Pyrene		10.1	24.72
PBA		0.64	389.73
Chrysene		7.1	35.36
Naphthalene		> 200	< 0.01
Anthracene		156	1.61
Benz[a]anthracene		20.8	12.56
Phenanthrene		160	1.53

5. Sample preparation detected by HPLC-FLD

The 0.5 g oil was diluted by 3 mL *n*-hexane and cleaned-up by using a XAD-2 column (37 cm × 1 cm i.d.), prepared by filling of XAD-2 resin of 5.56 g (dried at 60 °C for 2 h) into a Cleanert BAP-3 column. The column was rinsed with 5 mL of dichloromethane, and 5 mL of *n*-hexane, respectively before using it. The sample was added into the Cleanert BAP-3 column, and then eluted by 10 mL of *n*-hexane, and 5 mL of dichloromethane, respectively. The collected dichloromethane eluent was then

evaporated to near dryness on a rotary evaporator at 25 – 30 °C. The residue was dissolved in 1 mL of acetonitrile and ultrasonic for 10 seconds. The solution was stored at 4°C prior to use.

6. The optimization of the GNP- ICS assay

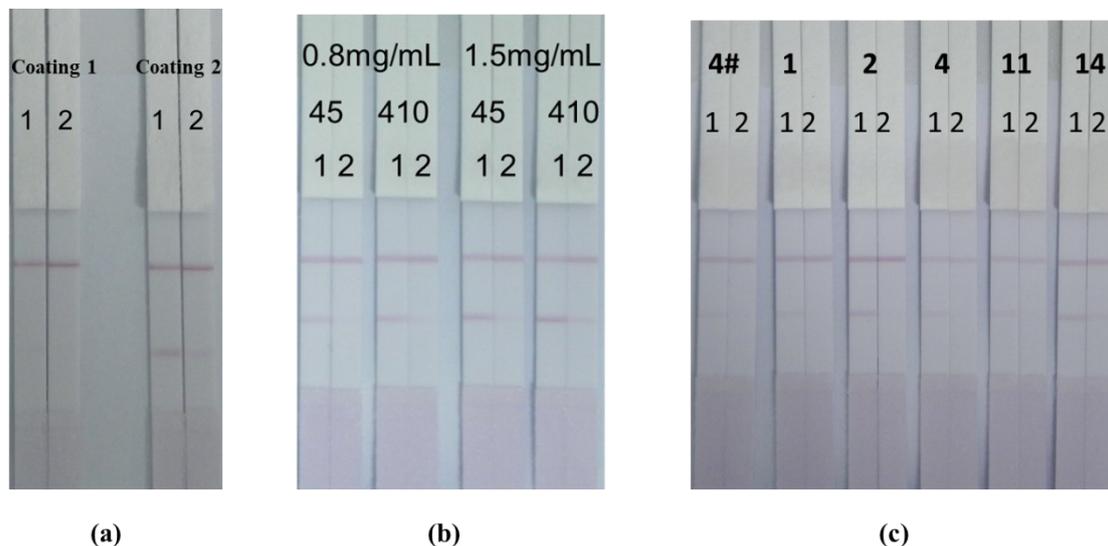


Figure S5. The optimization of the GNP- ICS assay. (a) Optimization of two coating antigen: coating 1, coating 2 represent the coating antigen PBA-OVA and PBA-BSA; 1, 2 represent the B[a]P concentration in 10% method-PBS at 0 and 25 ng mL⁻¹, respectively; (b) Optimization the concentration of coating antigen in PBS at 0.8 and 1.5 mg mL⁻¹, and optimization the concentration of GNPs-mAb, 45 = 5 µg mL⁻¹, 410 = 10 µg mL⁻¹; 1, 2 represent the B[a]P concentration at 0 and 100 ng mL⁻¹, respectively; (c) Optimiation of the GNPs-mAb probe resuspension. 4# = 0.02 M PBS containing 5% trehalose, 1% BSA and 0.05% NaN₃, 1= PVP, 2= PEG, 4 = Tween-20, 11= triton X-100, and 14= ON-870. 1, 2 represent the B[a]P concentration at 0 and 100 ng mL⁻¹, respectively.

7. Matrix effect on test strip

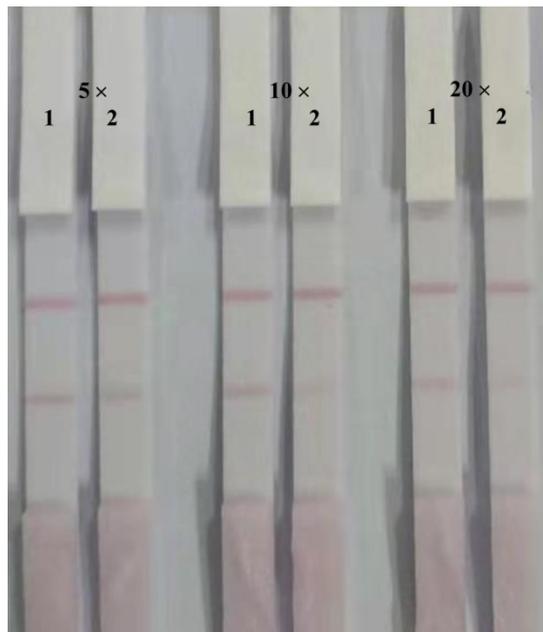


Figure S6. The matrix interference of oil extract on GNP-ICS. 5×,10×, 20× represent the oil extract were diluted with 10% DMSO-PBS at 5, 10, and 20 times; 1, 2 represent the B[a]P concentration at 0 and 500 ng mL⁻¹, respectively.

8. The GNP- ICS assay for detection of B[a]P in tea-seed oil

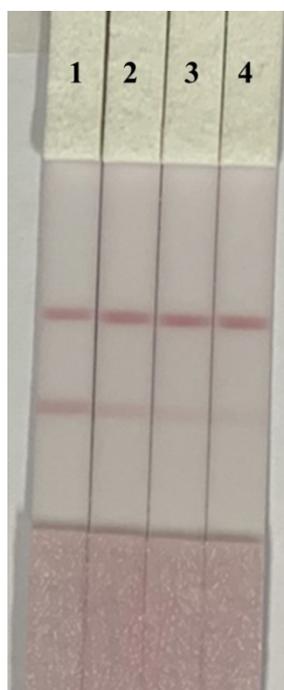


Figure S7. The GNP- ICS assay for detection of B[a]P in tea-seed oil sample. 1, 2, 3, and 4 represent the concentrations of B[a]P at 0, 200, 500, and 1000 ng mL⁻¹, respectively

Table S4. Chromatogram condition for the analysis of B[a]P by HPLC with fluorescence detector.

Instrument conditions	HPLC-fluorescence detector Waters 1525EF system		
Spectrum transmission microscope	Column	a reversed-phase C18 column (Agilent Zorbax Eclipse Plus) (4.6 ×250 mm, 5 μm)	
		Column temperature: 35 °C	
Mobile Phase	A: acetonitrile, 88%		
	B: water, 12%		
Fluorescence detector	Excitation wavelength at 384 nm		
	Emission wavelength at 406 nm		
Gradient Profile	Time (min)	Percentage A (%)	Percentage B (%)
	0	88	12
	47	88	12
Injection Volume	20 μL		
Flow velocity	1.0 mL min ⁻¹		

Table S5. The concentrations of B[a]P standard in acetonitrile detected by HPLC-FLD.

Sample	Concentration (ng mL ⁻¹)					
	SB	S1	S2	S3	S4	S5
B[a]P	0	0.5	1.0	5.0	10	20.0