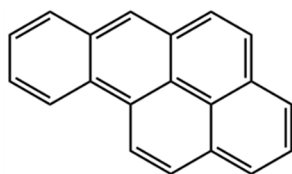
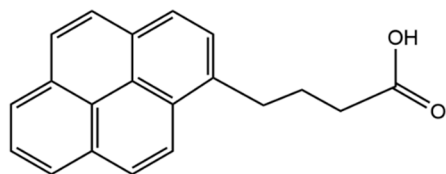


PYR



BaP



PBA

Figure S1 Structure of PYR, BaP and PBA

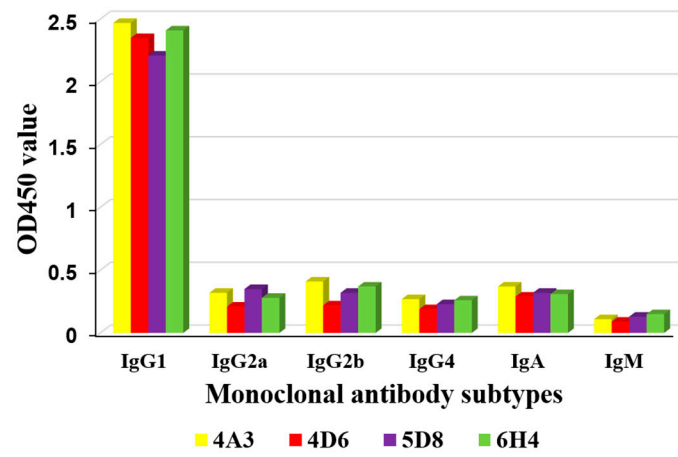


Figure S2. Subtype determination of four monoclonal antibodies

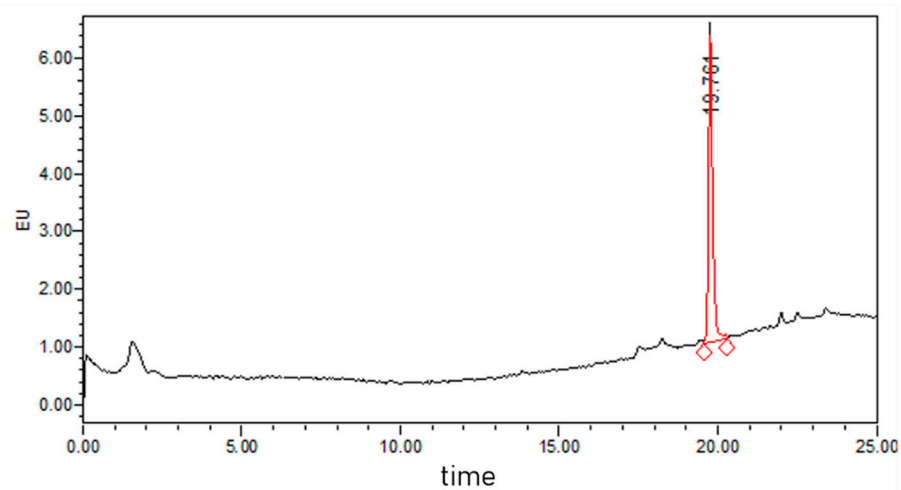


Figure S3 The liquid chromatogram spectrum of PYR in fish sample (10 ng/mL)

Table S1 Comparison of absorbance and inhibition rate of antigens with different coupling ratio on immunized mice

immunogen	diluted ratio of serum(1:x)	coating antigen (blank hole OD <sub>450</sub> value)			Inhibition rate ( B/B <sub>0</sub> )		
		B <sub>1</sub>	B <sub>2</sub>	B <sub>3</sub>	B <sub>1</sub>	B <sub>2</sub>	B <sub>3</sub>
A <sub>1</sub>	1:3200	1.034±0.026	1.381±0.023	1.576±0.035	0.57	0.52	0.45
A <sub>2</sub>	1:6400	1.487±0.043	1.765±0.017	1.922±0.028	0.47	0.42	0.36
A <sub>3</sub>	1:12800	1.642±0.021	1.956±0.040	2.127±0.052	0.39	0.28	0.21

Note: B: OD<sub>450</sub> nm value of wells containing PYR standard; B<sub>0</sub>: the OD<sub>450</sub> nm value of the wells that contained only assay buffer. Inhibition rates were calculated at the same drug concentration.

Table S2. Dilutions and IC<sub>50</sub> values of four monoclonal antibodies

Antibody	4A3	4D6	5D8	6H4
Antibody dilution (1:X)	20000	20000	16000	20000
IC <sub>50</sub> value(μg/L)	6.2	5.3	5.9	6.6

Table S3 Optimization of ELISA conditions

Optimization conditions	Parameter value	Blank hole OD <sub>450</sub> value	IC <sub>50</sub> (μg/L)
Coating concentration (μg/mL)	1	1.562±0.014	5.7±0.14
	2	2.001±0.021	4.2±0.11
	4	2.124±0.023	4.9±0.23
	8	2.201±0.047	6.2±0.15
The diluted concentration of mAb (1: X)	10000	2.371±0.051	6.7±0.21
	15000	2.187±0.044	5.8±0.23
	20000	2.068±0.035	4.4±0.14
	25000	1.847±0.021	5.2±0.31
Incubation time of primary antibody (min)	25	1.897±0.032	4.9±0.26
	35	2.003±0.018	3.7±0.22
	45	2.103±0.027	4.7±0.16
	55	2.116±0.037	5.3±0.20
Incubation time of HRP-IgG (min)	30	1.902±0.022	4.9±0.33
	40	2.032±0.035	4.1±0.18
	50	2.133±0.037	5.6±0.15
	60	2.157±0.039	6.1±0.17
Working concentration of HRP-IgG (1:X)	5000	2.268±0.032	5.6±0.27
	6000	2.037±0.028	4.3±0.21
	7000	1.832±0.035	4.9±0.18
	8000	1.597±0.033	4.6±0.14

Table S4 Cross-reactivity of 4D6 mAb with 16 polycyclic aromatic hydrocarbon drugs

Drugs	IC <sub>50</sub> (μg/L)	cross-reactivity (%)	R <sup>2</sup>
Pyrene	3.73	100	0.9975
Benzo[a]pyrene	9.71	38	0.9975
Fluoranthene	45.83	8	0.9972
Naphthalene	>373	<1	
Acenaphthene	>373	<1	
Acenaphthylene	>373	<1	
Fluorene	>373	<1	
Phenanthrene	>373	<1	
Anthracene	>373	<1	
Benz[a]anthracene	>373	<1	
Chrysene	>373	<1	
Benzo[b]fluoranthene	>373	<1	
Benzo[k]fluoranthene	>373	<1	
Dibenz[a,h]anthracene	>373	<1	
Benzo[g,h,i]perylene	>373	<1	
Indeno[1,2,3-cd] pyrene	>373	<1	