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Supporting Information

Immunological strip sensor for rapid determination of niacin in dietary supplements and foods

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1. Development of the ic-ELISA

Indirect ELISA was used to detect the titer of mouse tail antiserum (antibody), cell supernatant, and the screening of hybridoma cell lines. Then, the coating antigen was diluted with coating buffer (0.05 M carbonate buffer, pH 9.6), and added to wells of a 96 well plate (100 μL per well). It was then placed in an oven for 2 h at 37 ℃. After washing the 96 well plate with washing solution (0.05% (v/v) Tween-20 in 0.01 M PBS) three times, sealing buffer (0.05 M CBS containing 0.2% (m/v) gelatin) was added (200 μL per well), and placed in an oven for 2 h at 37 ℃. The plates were then washed three more times and dried for 40 min at 37 ℃ for subsequent experiments.

Next, we added antiserum (antibody) or cell supernatant and small molecule standard solution to a 96 well plate (50 μL per well) and incubated in an oven for 30 min at 37 ℃ and washed three washes, to remove non-specific antibody bind to the coating on the plate. Then, 100 μL of horseradish peroxidase labeled goat anti mouse immunoglobulin (IgG) was added to the 96 wells and incubated for 30 min at 37 ℃. After three washes, TMB (100 μL per well) was applied. After, the 96 well plates were incubated for 15 min at 37 ℃. Finally, we added termination buffer (2 M H_2SO_4, 50 μL per well) to stop the reaction and then measured the absorbance value of the liquid in the plate at 450 nm using an enzyme marker. In subsequent experiments, we set up three parallel experiments for the determination of each concentration. The absorbance values at 450 nm were recorded as B and B_0, representing the presence or absence of a competitor, respectively. The formula for the inhibition rate is:

$$\text{Inhibition rate (\%)} = 1 - B/B_0 \times 100\% \quad (1\text{-}3)$$

A series of niacin concentrations (0, 50, 100, 200, 500, 1000, 2000, and 5000 ng/mL) were measured and the corresponding ic-ELISA standard curves were constructed.

2. LC-MS/MS analysis

The liquid chromatograph (ACQUITY UPLC, Waters) is connected to the Quattro Premier XE system (Waters Quattro Primer XE system, USA) and can be used for compound structure identification and sample qualitative analysis. An ACQUITY UPLC BEH C_{18} column (100 mm × 2.1 mm i.d., 1.7 μm) was eluted with 0.1% formic acid (mobile phase A) and acetonitrile (mobile phase B). A linear gradient was employed for 5.0 min (from 98:2 to 60:40 mobile phase A: B), followed by 1 min at 20:80 and 1 min 2:98, and re-equilibrated for 1 min. The column temperature was 40 °C and the flow rate was 0.3 mL/min. The injection volume was 2 μL. The mass spectrometric detection and quantitation was conducted in negative ion mode using multiple reaction monitoring (MRM). Basic parameters included: capillary voltage, 3500 V; desolvation temperature, 400 °C; desolvation gas flow, 700 L/h; cone voltage, 20 V; e collision gas flow, 0.15 mL/min.

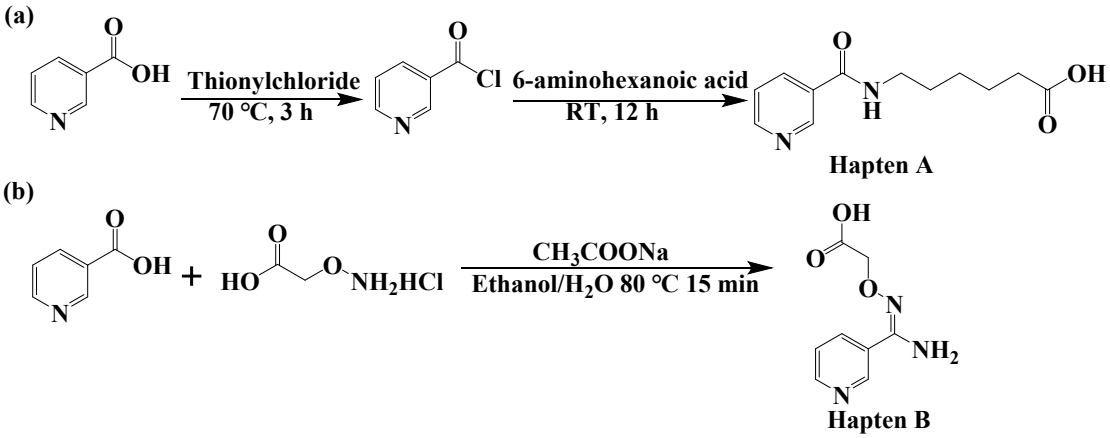


Fig. S1. (a) the synthetic route of the hapten A; (b) the synthetic route of the hapten B.

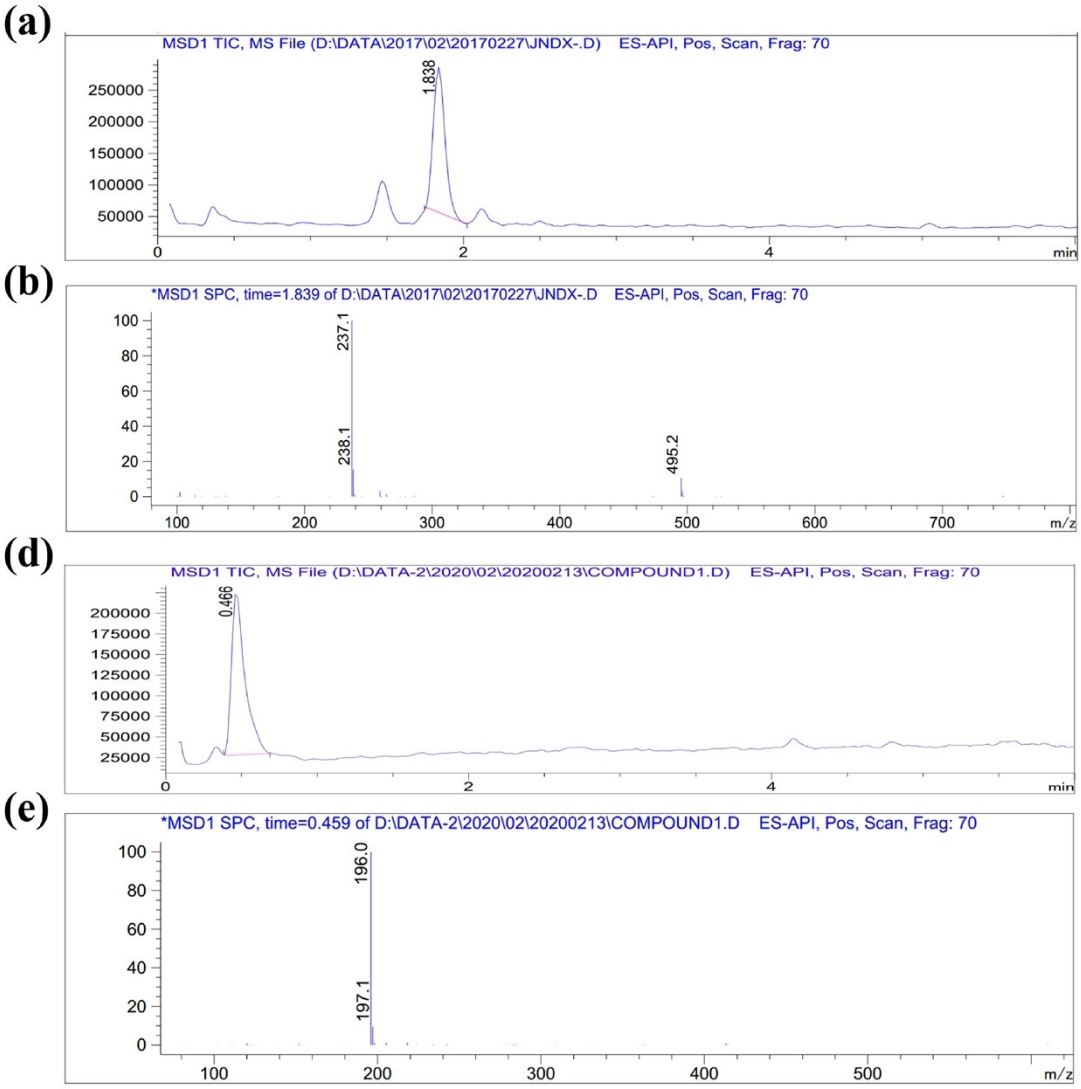


Fig. S2. (a) (b) The MS spectrum of hapten A, (c)(d) The MS spectrum of hapten B.

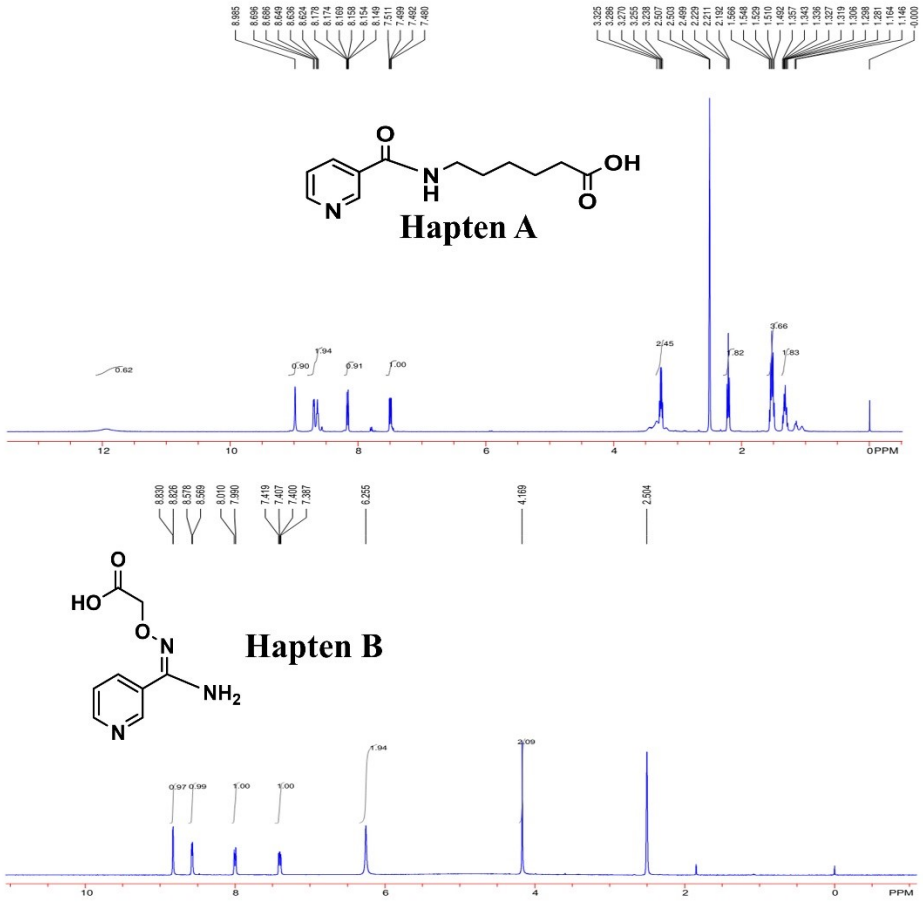


Fig. S3. The ^{1}H NMR spectrum of hapten A and hapten B, respectively.

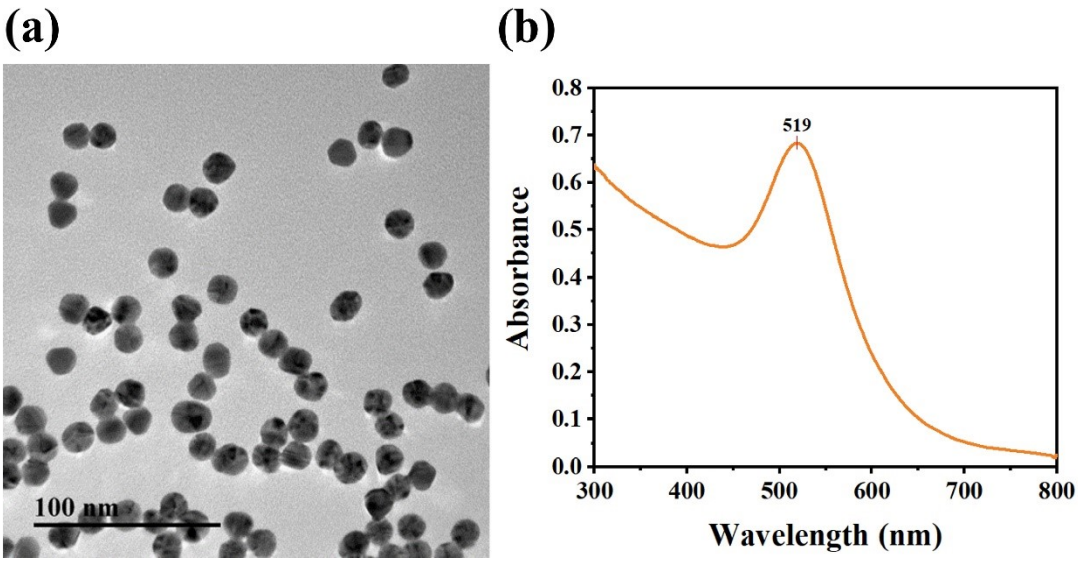


Fig. S4. Characterization of the colloidal gold. (a) TEM images; (b) UV-vis spectrum.

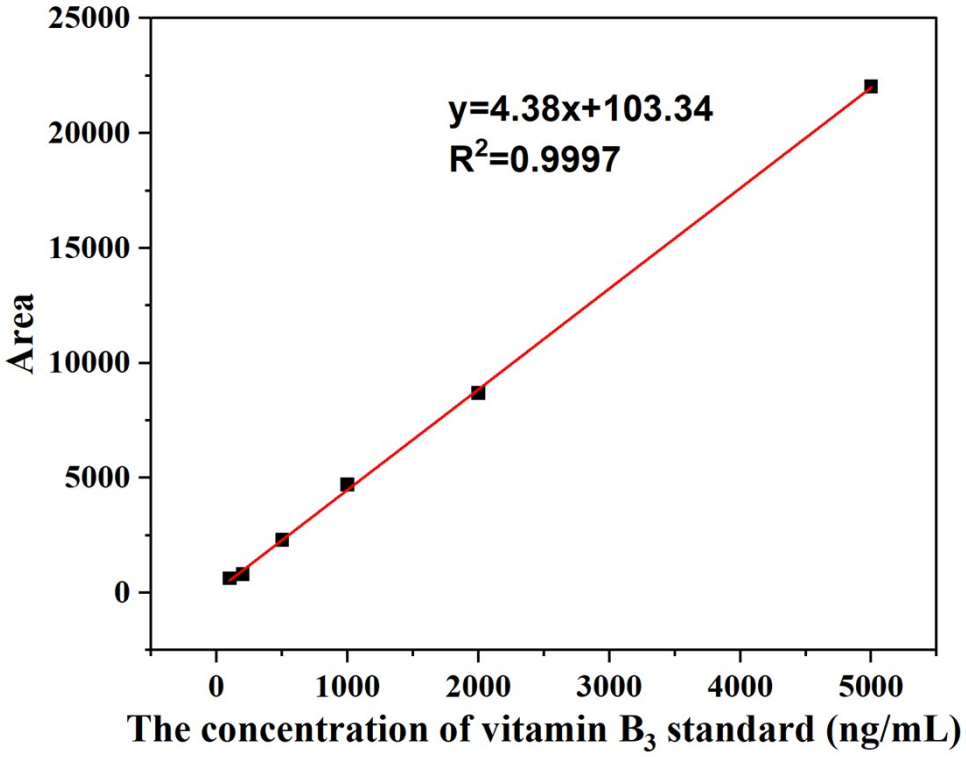


Fig. S5. The calibration curve for the HPLC of niacin.

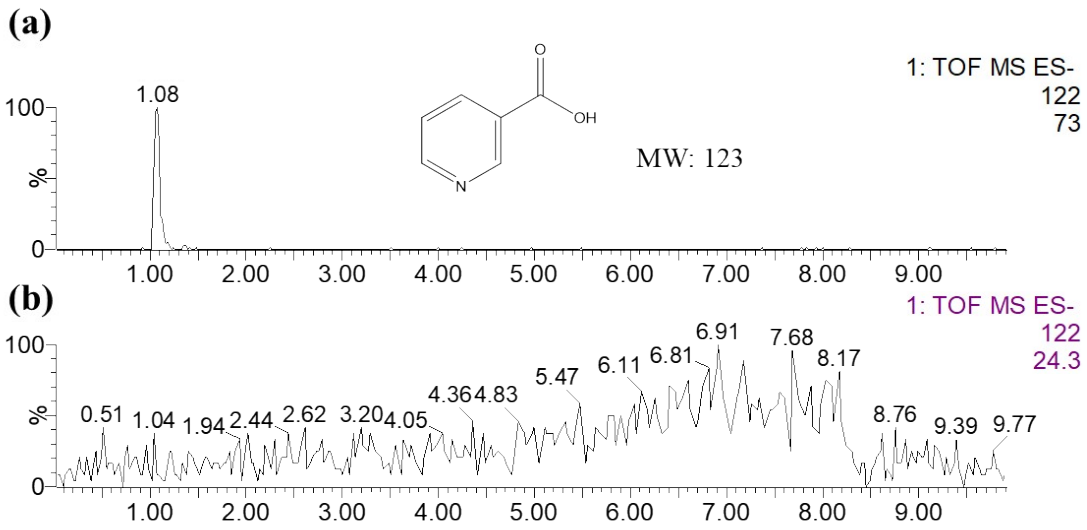


Fig. S6. The LC-MS/MS spectrum of sample. (a) The detection results of standard solution. (b) The detection results of blank complex vitamin B tablets.

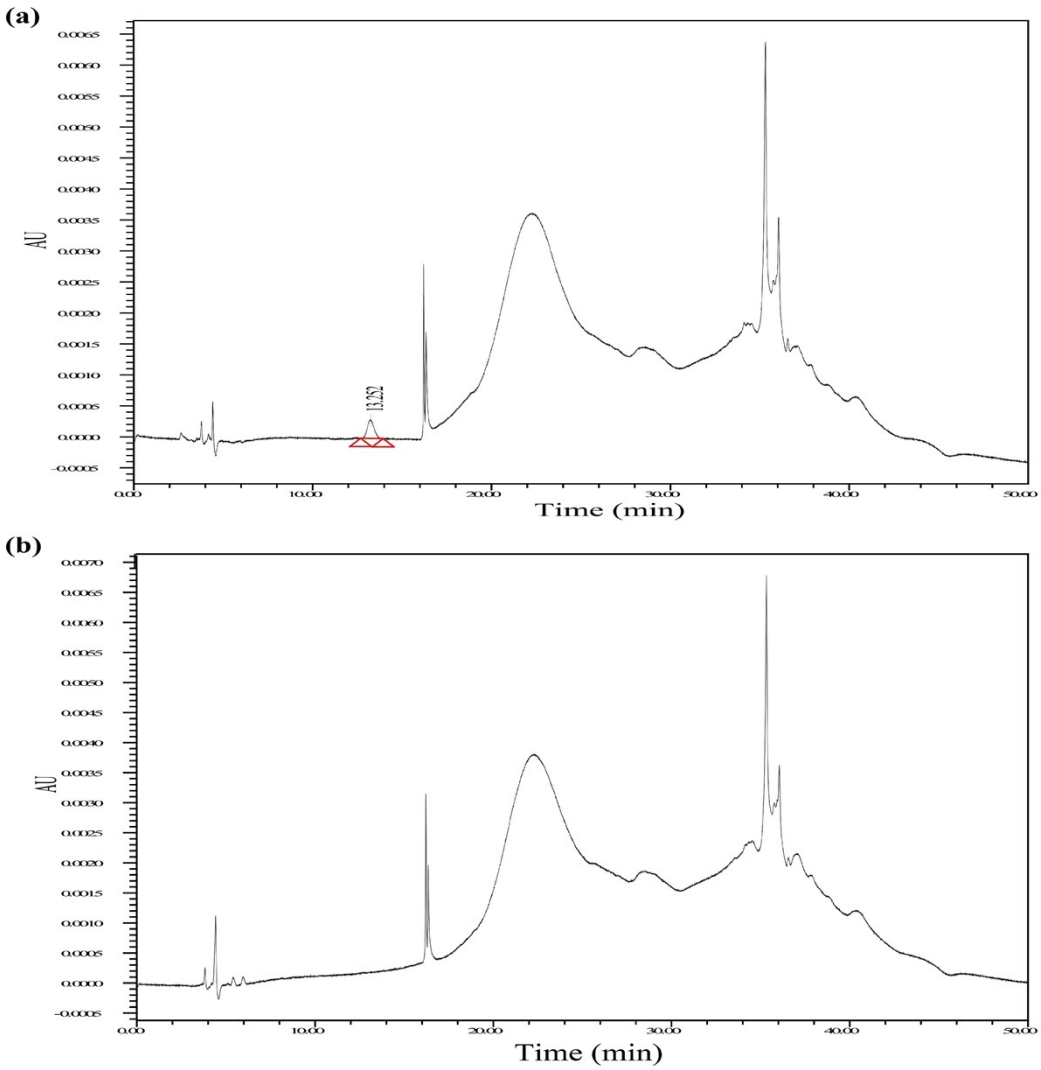


Fig. S7. The HPLC spectrum of sample. (a) The detection results of standard solution. (b) The detection results of milk powder.

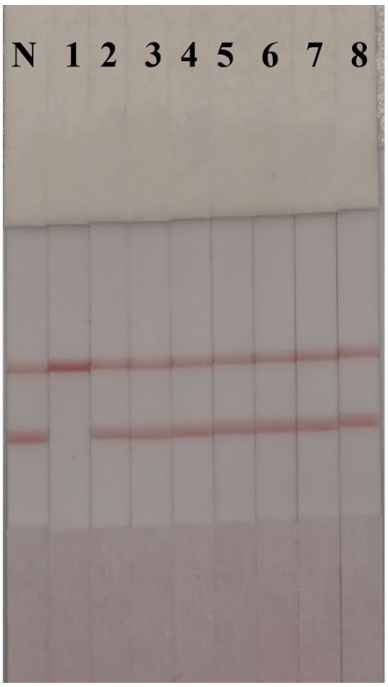


Fig. S8. Selection specificity analysis of the LFIA strips (50 μg/mL). N: PBS, 1: niacin; 2: VB1; 3: VB2; 4: VB5; 5: VB6; 6: VB7; 7: VB9; 8: VB12.

Table S1. Antiserum titer responses against various coating antigens.

Antiserum	Coating antigen[a]	Niacin	
		Titer[b] (× 10^3)	Inhibn[c] (%)
Hapten A-KLH	Hapten A-BSA	9.0	15.01
	Hapten B-OVA	3.0	NC[d]
	Hapten C-OVA	9.0	NC
	Hapten D-OVA	3.0	NC
Hapten B-BSA	Hapten B-OVA	9.0	4.87
	Hapten C-OVA	3.0	NC
	Hapten D-OVA	9.0	NC
Hapten C-BSA	Hapten B-OVA	3.0	10.09
	Hapten C-OVA	9.0	11.09
	Hapten D-OVA	9.0	NC
Hapten D-BSA	Hapten B-OVA	9.0	NC
	Hapten C-OVA	3.0	NC
	Hapten D-OVA	9.0	60.78

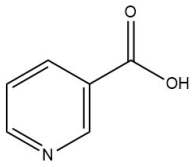
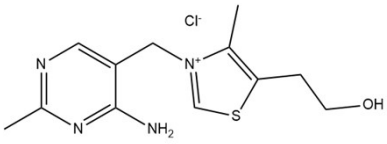
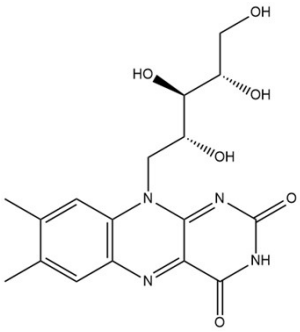
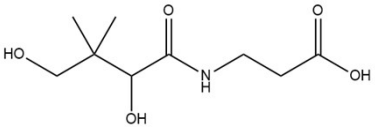
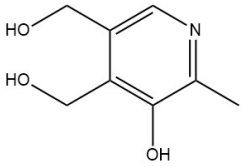
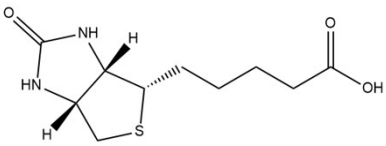
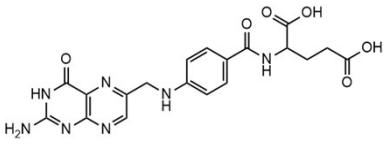
[a] The concentration of coating antigen was 0.3 mg/L for the detection of antisera.

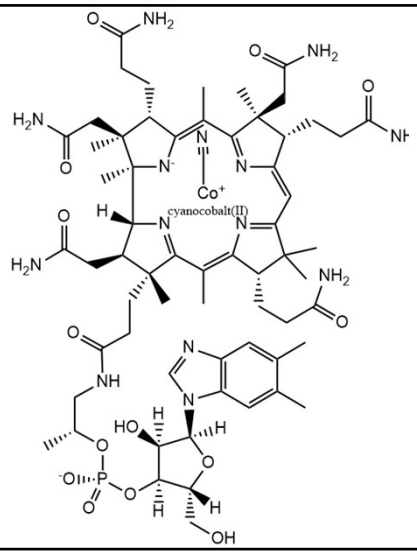
[b] Titer is defined as the dilution factor of antiserum.

[c] percentage inhibition was express as follow: Inhibition (%) =[1-(B/B_0)] × 100; the absorbance values obtained at 450 nm in the presence of competitor concentration (2000 ng/mL Niacin) and no competitor (maximum signal) were defined as B and B_0.

[d] NC: failed to calculate the inhibition rate.

Table S2. Cross-reactivity of the mAb with niacin (VB3) and other B vitamins using the ic-ELISA method (n=3)[a].

B Vitamins	structure	IC_{50} (ng/mL)	CR (%)
Niacin (VB3)		603.41	100.0
Thiamine (VB1)		>100000.0	＜0.02
Riboflavin (VB2)		>100000.0	＜0.02
Pantothenic acid (VB5)		>100000.0	＜0.02
Pyridoxine (VB6)		>100000.0	＜0.02
Biotin (VB7)		>100000.0	＜0.02
Folic acid (VB9)		>100000.0	＜0.02

Cobalamins (VB12)		>100000.0	＜0.02