

supplementary materials

Design of Novel haptens and Development of Monoclonal Antibody-based Immunoassays for the Simultaneous Detection of Tylosin and Tilmicosin in Milk and Water Samples

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This material includes: The synthesis route of haptens (Figure S1), the full scan mass spectra of haptens (Figure S2), UV absorption curve of conjugates (Figure S3), optimization of icELISA working condition (Table S1, Figure S4), the isotype of mAb L02 (Figure S5), HPLC-MS/MS calibration (Figure S6), the linear regression analysis of between icELISA with HPLC-MS/MS (Figure S7).

Antigen preparation. The synthesis route and full scan mass spectra of hapten TYL-CHO was showed in figure S1, figure S2. Then the hapten were coupled to BSA as the immunogen and to OVA as the coating antigen by active ester method. The successful conjugations were confirmed by UV-visible spectra (Figure S3).

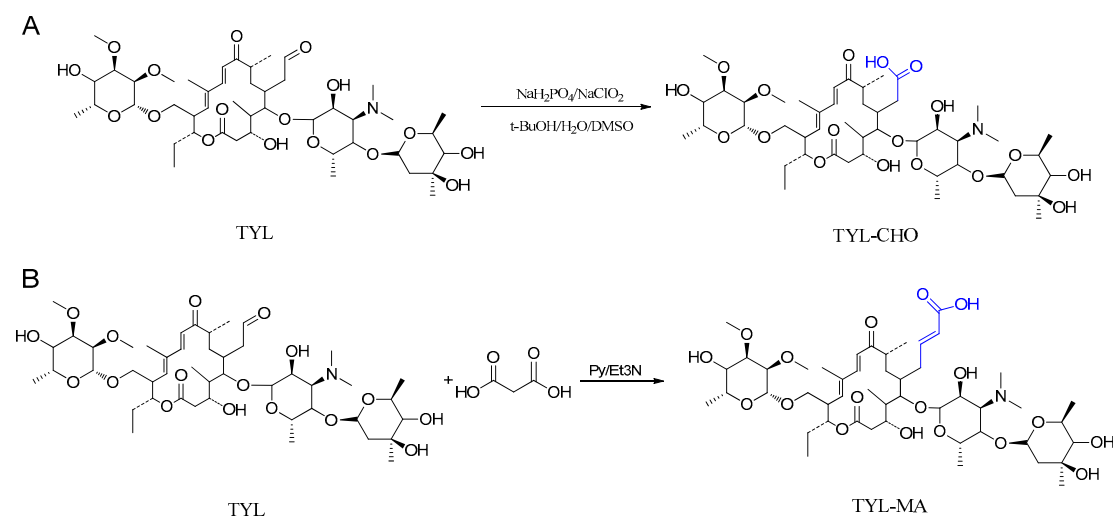


Figure S1. The synthesis route of haptens.

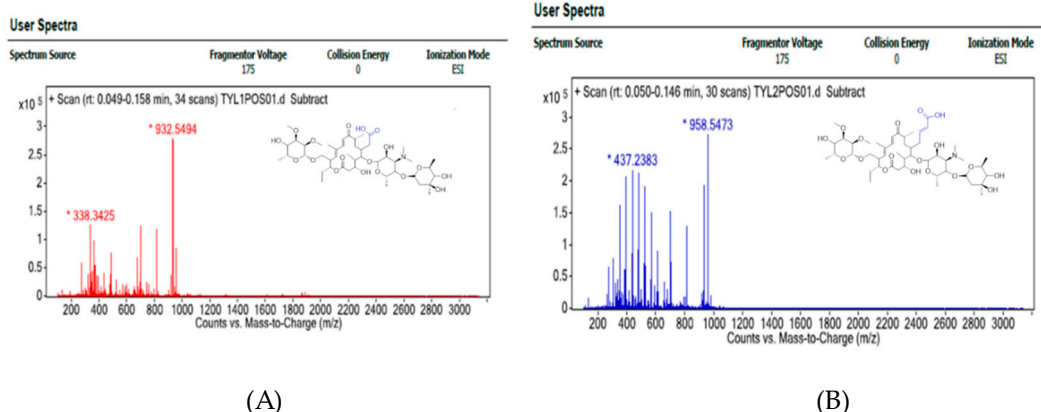


Figure S2. Full scan mass spectra of four haptens. (A: TYL-CHO, $[M+H]^+$ m/z 932.5494; B: TYL-MA, $[M+H]^+$ m/z 957.53)

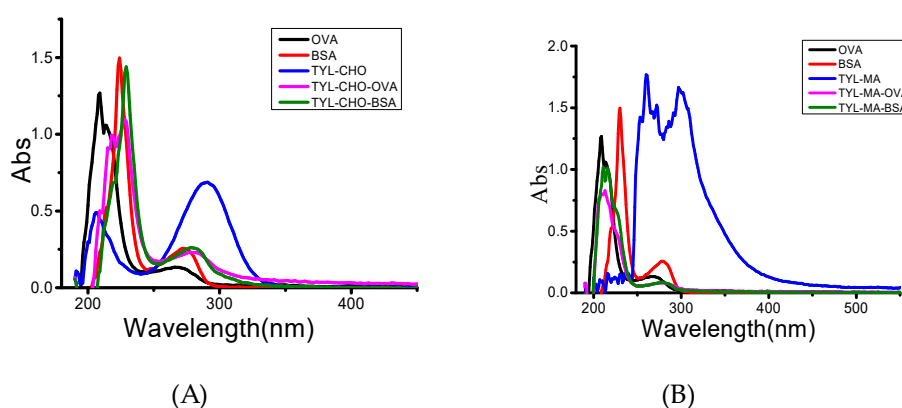
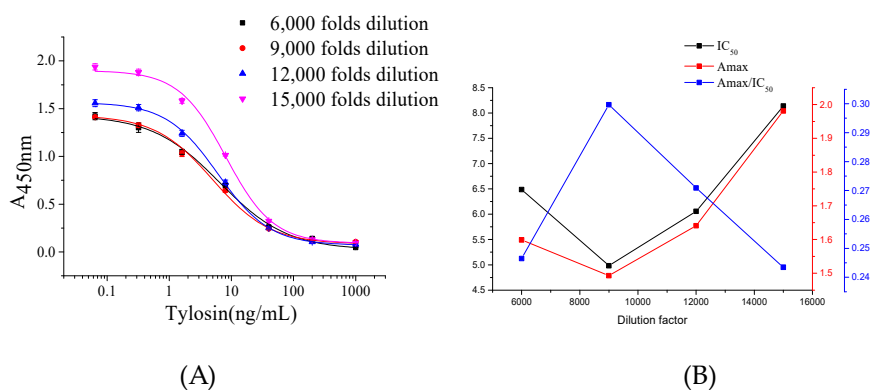
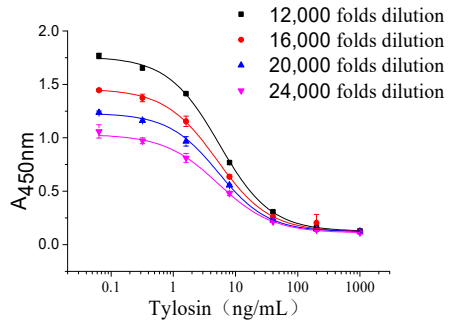


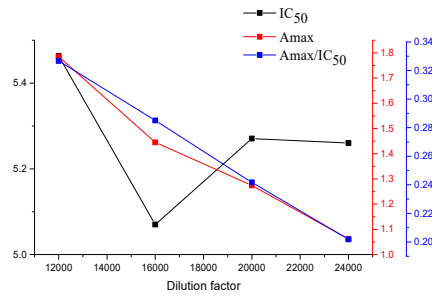
Figure S3. The UV-vis spectral data of conjugates, hapten and proteins. (A: TYL-CHO; B: TYL-MA)

Optimization of icELISA working condition (Figure S4, Table S1). Several parameters in icELISA were optimized, including the concentration of antibody, coating antigens and HRP-IgG, working buffer (ionic strength, pH value and the concentration of tween-20) and reaction time. According to the chessboard titration and dose-response curves, the maximum absorbance value (A_{max}) and half maximal inhibitory concentration (IC_{50}) were calculated. The optical conditions were confirmed by A_{max} , A_{max}/IC_{50} and IC_{50} . A condition with higher value of A_{max}/IC_{50} and lower value of IC_{50} were selected to use in assay.

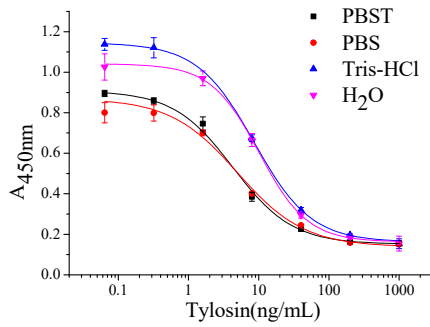




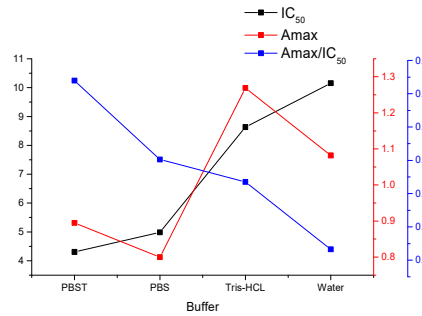
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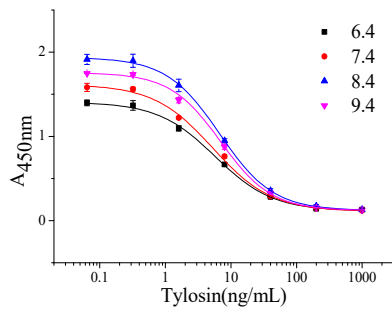
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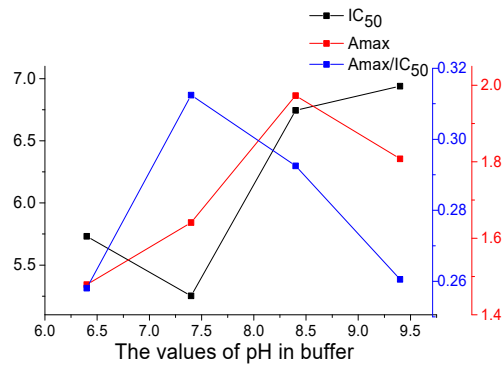
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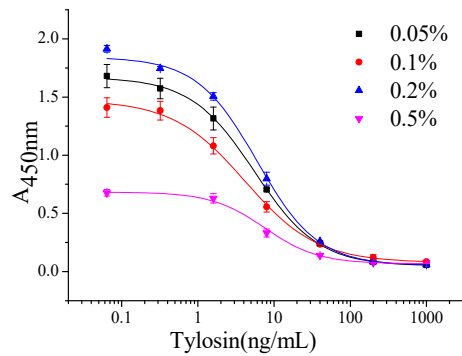
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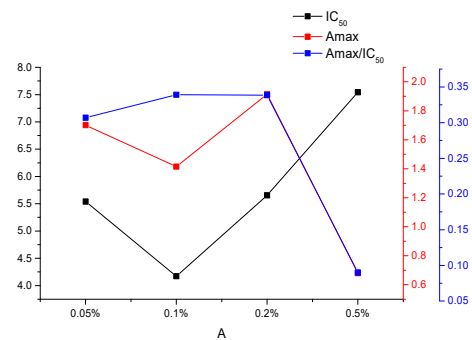
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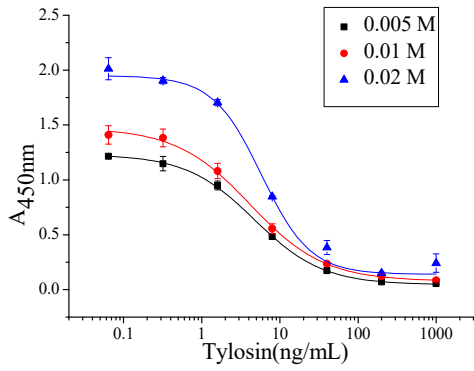
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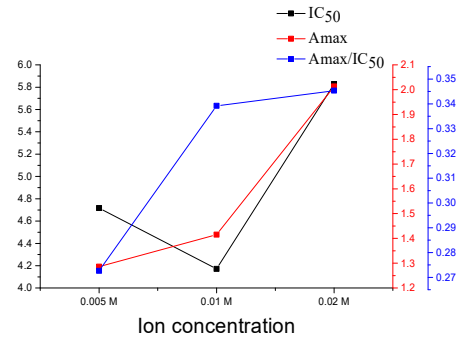
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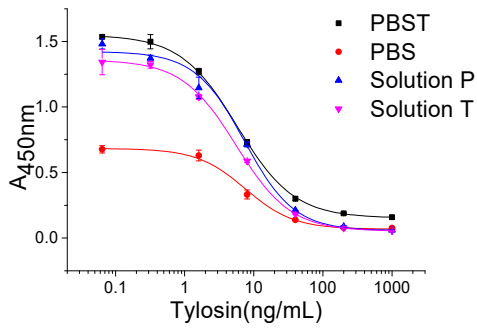
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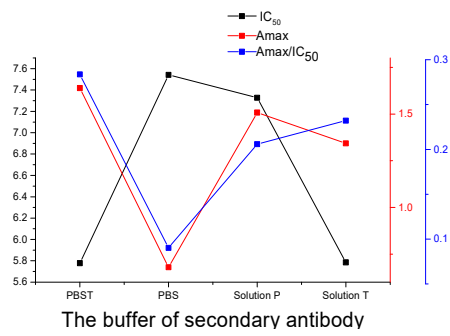
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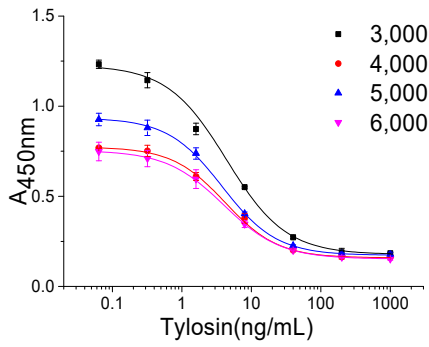
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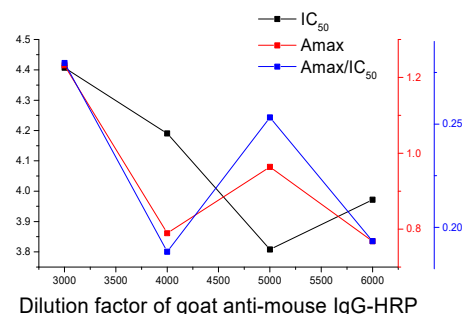
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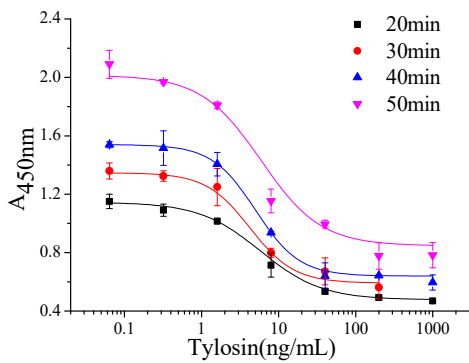
(N)



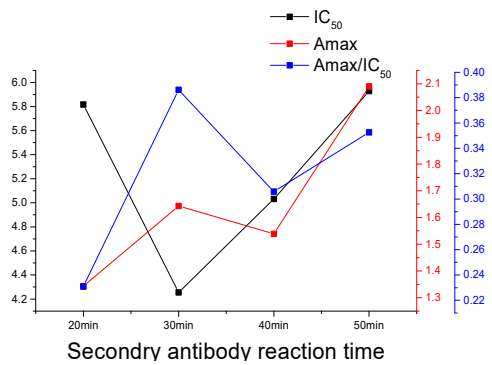
(O)



(P)



(Q)



(R)

Figure S4. Optimization of icELISA working condition. Concentration of coating antigen (A, B), concentration of antibody (C, D), working buffer (E, F), pH of working buffer (G, H), Tween-20 content of buffer (I, J), ion concentration of buffer (K, L), the buffer of secondary antibody (M, N), dilution factor of goat anti-mouse IgG-HRP (O, P), secondary antibody reaction time (Q, R). A, C, E, G, I, K, M, O, Q are calibration curves, and B, D, F, H, J, L, N, P, R are trend curves of A_{MAX} , IC_{50} and A_{MAX}/IC_{50} .

The isotype of mAb L02 (Figure S5). The mAb L02 was diluted as 100 ng mL^{-1} by sample diluent. The commercial strip showed that multiple bands detected but the darkest band was the isotype of the most abundant antibody in the sample.

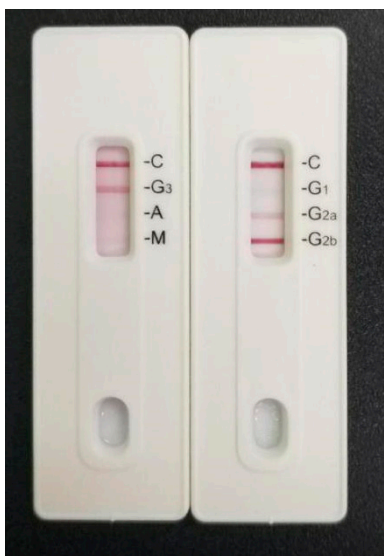
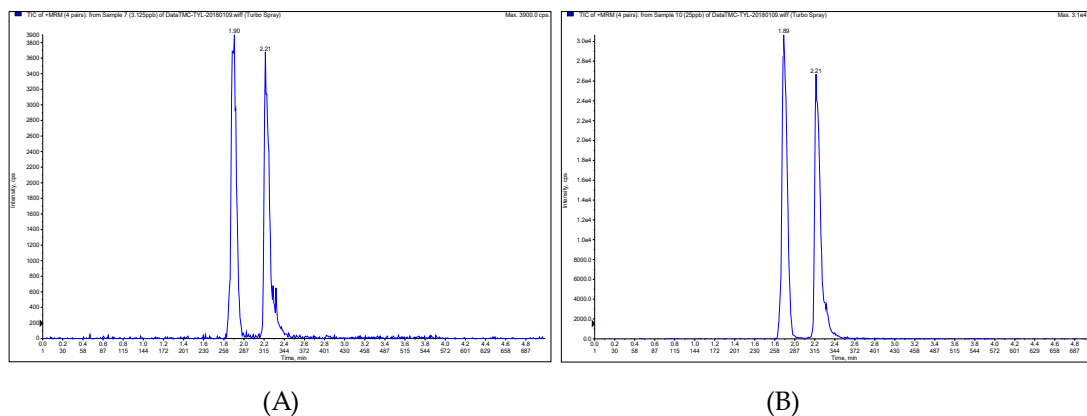
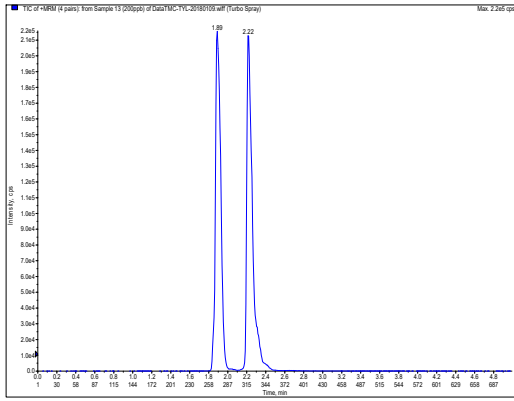


Figure S5. The isotype of mAb L02





(C)

Figure S6. Mass spectra of mixture standard of tylosin and tilmicosin. (A, B, C: the concentration of mixture standard was 3.125 ng mL⁻¹, 25 ng mL⁻¹, 200 ng mL⁻¹.)

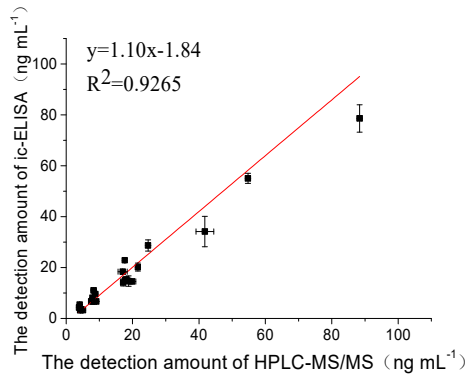


Figure S7. The linear regression analysis of between icELISA with HPLC-MS/MS

Table S1. The result of chessboard method for icELISA.

Dilution of antiserum	Dilution of coating antigen			
	6,000	9,000	12,000	18,000
1,000	3.42	3.42	3.08	2.10
2,000	3.15	2.94	2.37	1.53
4,000	3.01	2.45	1.68	0.98
8,000	2.58	1.62	1.00	0.56
16,000	1.69	0.95	0.56	0.33
32,000	1.06	0.53	0.33	0.20
64,000	0.61	0.33	0.20	0.13
0	0.08	0.06	0.06	0.07