Electronic Supplementary Material (ESI) for Analytical Methods.

Supporting information:

Development of a sensitive monoclonal antibody-based ELISA for the determination of a β-adrenergic agonist brombuterol in swine meat, liver and feed samples

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The processes of the monoclonal antibody production

1. Immunization

Seven female BALB/c mice (6-8 weeks) were immunized subcutaneously with brombuterol-BSA conjugate. The immunization procedures were as follows. Brombuterol-BSA was dissolved in physiological saline and then sufficiently mixed with equal volume of Freund's adjuvant to form an emulsion. The mice were subcutaneously immunized and each mouse was received 100 μg immunogen. Two weeks after the initial immunization, the mice were received the second immunization with the same amount of immunogen accompanied with incomplete Freund's adjuvant. The interval between the two/third and third/fourth immunization was two weeks. Ten days after the fourth immunization, the blood of each immunized mouse was separately collected from the caudal vein. The titer and affinity of the sera obtained from the mice were tested by an ic-ELISA in which brombuterol-OVA was used as a coating antigen (1 μg mL^{-1}), the mice serum was diluted at the ratio of 1:1,000–1:100,000 and brombuterol was used as the competitor (the detailed procedures of the ic-ELISA were given in the section of "Procedures of indirect competitive ELISA"). After 14 days of the fourth immunization, two mice with the lowest IC_{50} values (e.g. the highest affinity recognition with brombuterol) were intraperitoneally received the fifth immunization with 100 μg of the immunogen dissolved in physiological saline without adjuvant. Three days later, the spleen cells from these two mice were collected for cell fusion.

2. Cell fusion

The generation of hybridomas was performed as described elsewhere (Wang et al.,

2009). The spleen cells collected from immunized mice were fused with SP2/0 cells at the initial ratio of 10:1 at 37°C in a water bath. 50% of PEG_{4000} was used as a fusogenic agent. After the fusion process, the mixture of cells and RPMI-1640 medium were spread in 96-well cell culture plates in which hypoxanthine–aminopterin–thymidine (HAT) medium containing 15% fetal calf serum were previously added for hybridomas selection and cell nutrition. The 96-well culture plates were incubated in cell culture incubator with the atmosphere of 5% CO_2 and the temperature of 37°C.

3. Hybridoma selection and cloning

Ten days after the incubation of cells, the supernatants from every well of the culture plates were collected and tested by an ic-ELISA. Every 96-well culture plate for cell culture and microtiter plate for ELISA was numbered to prevent confuse. The 96-well microtiter plates were coated with brombuterol-OVA (100 μL $well^{-1}$, 5 μg mL^{-1} in coating buffer) overnight at 4 °C. The plates were washed with PBST three times and then blocked with 1% casein (140 μL $well^{-1}$ dissolved in PBS) at room temperature for 1 h. The plates were washed again as before, then each well of the plates received 50 μL of hybridomas supernatants from each well of the corresponding numbered culture plates and then were incubated at room temperature for 1 h. After washing, HRP-GaMIgG diluted in PBS (100 μL $well^{-1}$) was added to the plates and incubated for 2 h. After the plates were washed, the substrate solution (100 μL $well^{-1}$) was added to the plates and shaken for 15-20 minutes. Sulfuric acid (60 μL $well^{-1}$) was added to the plate to stop the enzymatic reaction. The absorbance of each well of the plates was read with ELISA reader at 450 nm. The hybridomas capable of secreting mAb to the

culture supernatants which were tested by ic-ELISA with the high absorbance value (top 5%) were expanded. The culture supernatants from the expanded hybridomas were tested by ic-ELISA again. Only those which secreted antibodies specific to brombuterol were selected and subcloned further. After subcloning 2 times by the stepwise dilution, the cell clones which were able to secret the antibody against brombuterol stably were finally expended, frozen overnight at −80 °C and stored in liquid nitrogen.

Five BALB/c male mice were used for antibody production. Each mouse was intraperitoneally injected with 0.5 mL of pristane. A week later, each mouse was intraperitoneally injected with 8×10^5 hybridoma cells. After 10 days, the ascites from the mice were collected, mixed with equal volume of glycerol (containing 0.02% of NaN_3) and stored at −20 °C until use.

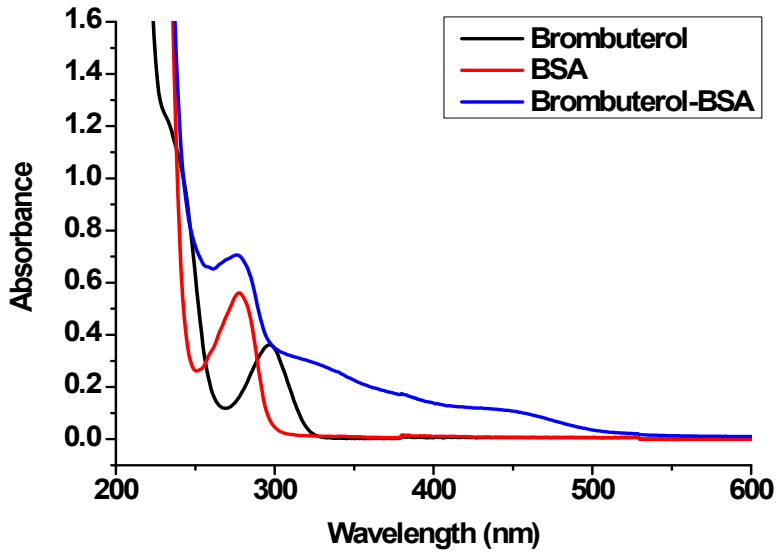

Fig. S1. UV-visible spectra of BSA, brombuterol and the brombuterol–BSA conjugate