Salbutamol ELISA Test Kit

1. Principle

The test kit is based on the competitive enzyme immunoassay for the detection of Salbutamol in the sample. The coupling antigen is pre-coated on the micro-well stripes. The Salbutamol in the sample and the coupling antigens pre-coated on the micro-well stripes compete for the anti-Salbutamol antibodies. After the addition of the enzyme conjugate, the TMB substrate is added for coloration. The optical density (OD) value of the sample has a negative correlation with the Salbutamol in it. This value is compared to the standard curve and the Salbutamol residues is subsequently obtained.

2. Technical specifications

Sensitivity: 0.5 ppb

Detection limit

Tissue ..... 0.5 ppb
Urine, serum ..... 0.5 ppb
Feed ..... 50 ppb

Recovery rate

Urine, serum ..... 90±10%
Tissue ..... 80±10%
Feed ..... 80±15%

Cross-reaction rate

Salbutamol 100%
Terbutalin ..... < 1%
Clenbuterol ..... < 13%
Ractopamine ..... < 1%

3. Components

1） Micro-well strips: 12 strips with 8 removable wells each
2） 6× standard solution (1 mL each): 0 ppb, 0.5 ppb, 1.5 ppb, 4.5 ppb, 13.5 ppb ,40.5 ppb
3） Enzyme conjugate (12 mL) ..... red cap
4） Antibody working solution (7 mL) ..... blue cap
5） Substrate A solution (7 mL) ..... white cap
6） Substrate B solution (7 mL) ..... black cap
7） Stop solution (7 mL) ..... yellow cap
8） 20x concentrated washing buffer (40 mL) ..... white cap
9） 2x concentrated redissolving solution (50 mL) ..... transparent cap

4. Materials required but not provided

1) **Equipments:** microplate reader, printer, homogenizer, nitrogen-drying device, vortex, oscillator, centrifuge, measuring pipets, balance (a sensibility reciprocal of 0.01 g)
2) **Micropipettors:** single-channel 20-200 µL and 100-1000 µL, and multi-channel 250 µL;

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3) **Reagents:** Acetonitrile (CH_3CN), NaOH, ethyl acetate, N-hexane, HCl (approx 36.5%), isopropanol

5. Sample pre-treatment

Instructions (The following points must be dealt with before the pre-treatment)

1) Only the disposable tips can be used for the experiments and the tips must be changed when used for absorbing different reagents;
2) Before the experiment, each experimental utensil must be clean and should be re-cleaned if necessary, in order to avoid the contamination that interferes with the experimental results.

Solution preparation before sample pre-treatment:

1) 1 M HCl: dissolve 8.6 mL HCl (approx 36.5%) in deionized water to 100 mL
2) 1 M NaOH: dissolve 4 g NaOH in deionized water to 100 mL
3) The 2× concentrated redissolving solution is mixed with deionized water at 1:1 (1 mL concentrated redissolbing solution + 1 mL deionized water, for redissolving) and 1:3(for extracting sample).

5.1 Samples preparation

a) Urine and serum

Take 50 µL clear urine or serum, directly detect it (If urine and serum are muddy, must filter or centrifuge at 4000 r/min at 15 ℃for 10 min, then take clear urine and serum). Store at frozen if don't use.

Some interference in urine, we recommend 1 ppb as cut off value of positive sample.

b) *First* method of recovery (liver, pork)

1. Weigh 2 ± 0.05 g sample, add 1 mL 0.5× diluted redissolving solution(dilluted at 1:3), mix properly, then add 1 mL isopropanol, vortex for 5 min, centrifuge at 4000 r/min at room temperature (20-25 ℃) for 10 min.
2. Take 3 mL supernatant, add 50 µL 1 M NaOH, mix properly, then add 7 mL Ethyl acetate solution, shake for 5 min, centrifuge at 4000 r/min at room temperature (20-25 ℃) for 10 min, take all supernatant, blow to dry with nitrogen or air at 50 ℃.
3. Dissolve residues in 1 mL 1×diluted redissolving solution(dilluted at 1:1),add 1ml N-hexane ,shake properly fpr 30s, centrifuge at 4000 r/min room temperature(20-25 ℃) for 10 min.
4. Take 50 µL for analysis.

Fold of dilution of sample: 1

***Second* method of recovery** (feed)

1. Take 2 ± 0.05 g grinded sample, add 2 mL 1 M HCl, 16 mL deionized water, homogenize.
2. Vortex for 3 min, shake for 5 min with oscillator .
3. Centrifuge at 4000 r/min for 15 min, take the supernatant (upper layer) , add 1 mL 1 M NaOH, adjust PH value to 6-8.
4. Centrifuge at 4000 r/min for 15 min(If it's not clear, should be centrifuged at higher speed).

5. Dilute supernatant with diluted redissolving solution at 1:9(100 µL supernatant + 900 µL diluted redissolving solution).
6. Take 50 µL for further analysis.

Fold of dilution of sample: 100

6. ELISA procedures

1. Bring test kit to the room temperature (20-25 ℃) for at least 30 min, note that each reagent must be shaken evenly before use; put the required micro-well strips into plate frames. Re-sealed the unused microplate, stored at 2-8 ℃, not frozen;
2. Solution preparation: dilute 40 mL of the concentrated washing buffer (20 × concentrated) with the distilled or deionized water to 800 mL (or just to the required volume) for use.
3. Numbering: number the micro-wells according to samples and standard solution; each sample and standard solution should be performed in duplicate; record their positions;
4. Add 50 µL of the sample or standard solution to separate duplicate wells, and add antibody working solution, 50 µL/well, seal the microplate with the cover membrane, and incubate at 37 ℃for 30 min;
5. Pour the liquid out of microwell, wash the microplate with the washing buffer at 250 µL/well for four to five times. Each time soak the well with the washing buffer for 15 sec, flap to dry with absorbent paper (if there are the bubbles after flapping, cut them with the clean tips).
6. Add 100 µL enzyme conjugate into each well, seal the microplate with the cover membrane, and incubate at 37 ℃ for 30 min, continue as described in 5.
7. Coloration: add 50 µL of substrate A solution and 50 µL B solution into each well. Mix gently by shaking the plate manually, and incubate at 37 ℃for 15 min at dark for coloration;
8. Determination: add 50 µL stop solution into each well. Mix gently by shaking the plate manually. Set the wavelength of the microplate reader at 450 nm to determine the OD value of every well. (Recommend to read the OD value at the dual-wavelength 450/630 nm within 5 min).

8. Result judgment

There are two methods to judge the results; the first one is the rough judgment, while the second is the quantitative determination. Note that the OD value of the sample has a negative correlation with Salbutamol concentration in the sample.

8.1 Qualitative determination

The concentration range (ng/mL) can be obtained from comparing the average OD value of the sample with that of the standard solution. Assuming that the OD value of the sample Ⅰ is 0.313, and that of the sample Ⅱ is 1.032, the OD value of standard solutions is: 1.892 for 0 ppb, 1.501 for 0.5 ppb, 1.175 for 1.5 ppb, 0.751 for 4.5 ppb, 0.421 for 13.5 ppb ,0.198 for 40.5 ppb, accordingly the concentration range of the sample Ⅰ is 13.5 to 40.5 ppb, and that of the sample Ⅱ is 1.5 to 4.5 ppb.

8.2 Quantitative determination

The mean values of the absorbance values obtained for the average OD value (B) of the sample and the standard solution divided by the OD value (B0) of the first standard solution (0 standard) and subsequently multiplied by 100%, that is

$$\text{Percentage of absorbance value} = \frac{B}{B0} \times 100\%$$

B—the average (double wells) OD value of the sample or the standard solution

B0—the average OD value of the 0ng/mL standard solution

Draw the standard curve with the absorption percentages of the standard solutions and the semilogarithm values of the Clenbuterol standard solutions (ng/mL) as Y- and X-axis, respectively. Read the corresponding concentration of the sample from the standard curve by incorporating its absorption percentage into the standard curve. The resulting value is subsequently multiplied by the corresponding dilution fold, finally obtaining the Salbutamol concentration in the sample.

Using the professional analyzing software of this kit will be more convenient for the accurate and rapid analysis of a large amount of samples. (Please contact us for this software)

9. Precautions

1. Bring all reagents and micro-well strips to the room temperature (20-25 ℃) before use.
2. Return all reagents to 2-8 ℃ immediately after use.
3. The reproducibility of the ELISA analysis, to a large degree, depends on the consistency of plate washing. The correct operation of plate washing is the key point in the procedures of ELISA.
4. For the incubation at constant temperatures, all the samples and reagents must avoid light exposure, and each microplate should be sealed by the cover membrane.
5. The room temperature below 20 ℃ or the temperature of the reagents and the samples being not returned to the room temperature (20-25 ℃) will lead to a lower standard OD value.
6. Dryness of the microplate in the washing process will be accompanied by the situations including the non-linear standard curves and the undesirable reproducibility; So continue to next step immediately after washing.
7. Mix evenly, otherwise there will be the undesirable reproducibility.
8. The stop solution is the 2 M sulfuric acid solution, avoid contacting with the skin.
9. Do not use the kit exceeding its expiry date. The use of diluted or adulterated reagents from the kits will lead to the changes in the sensitivity and the detecting OD values. Do not exchange the reagents from the kits of different lot numbers to use.
10. Put the unused microplate into an auto-sealing bag to re-seal it. The standard substance and the colourless color former is light sensitive, and thus they cannot be directly exposed to the light.
11. Discard the colouration solution with any color that indicates the degeneration of this solution. The detecting value of the 0 standard solution (0 ppb) of less than 0.5 indicates its degeneration.

9. Storage and expiry date

Storage: store at 2-8 ℃, not frozen.

Expiry date: 12 months; date of production is on box.

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Green Earth depends on everyone' efforts

"Build of green Earth needs the cooperation of you and me"

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