β-agonists ELISA Test Kit

1. Principle

This test kit is based on the competitive enzyme immunoassay for the detection of β-agonists in the pork and liver. The coupling antigen is pre-coated on the micro-well stripes. The β-agonists in the sample and the coupling antigens pre-coated on the micro-well stripes compete for the anti-β-agonists 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 β-agonists in the sample. This value is compared to the standard curve and the β-agonists residues is subsequently obtained.

2. Technical specifications

Sensitivity: 0.1 ppb

Detection limit

Tissue ..... 0.1 ppb
Urine, serum ..... 0.1 ppb
Feed ..... 10 ppb

Recovery rate

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

Cross-reaction rate

Clenbuterol 100%
Salbutamal 61%
Terbutalin 52%
Mabuterol 45%
Brombuterol 45%
Carbuterol 50%

3. Components

1） Micro-well strips: 12 strips with 8 removable wells each
2） 6× standard solution (1 mL each): 0 ppb, 0.1 ppb, 0.3 ppb, 1 ppb, 5 ppb ,10 ppb
3） Enzyme conjugate (7 mL) ..... red cap
4） Antibody working solution (10 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

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, 100-1000 μL, and multi-channel 250 μL;

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3) Reagents: Acetonitrile (CH_3CN), NaOH, ethyl acetate, HCI (approx 36.5%)

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.
3) Tissue samples includes:pork;beef;sheep;and so on of musle;kidney;liver;retina and liver tissue.

Solution preparation before sample pre-treatment:

1) 0.1 M HCI: dissolve 0.86 mL HCI (approx 36.5%) in deionized water to 100 mL.
2) 1 M HCI: dissolve 8.6 mL HCI (approx 36.5%) in deionized water to 100 mL.
3) 1 M NaOH(for feed sample): dissolve 4 g NaOH in deionized water to 100 mL.
4) 0.1 M NaOH(for tissue samples): dissolve 0.4 g NaOH in deionized water to 100 mL.
5) CH_3CN-0.1 M HCI solution(for tissue samples): $V_{CH3CN} : V_{HCl}$ =84:16

5.1 *Urine and serum*

Take 20 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 enviroment if don't use.

5.2 *Tissue* (liver, pork, etc.)

1. Weigh 2±0.05 g sample, add 6 mL CH_3CN-0.1 M HCI solution, vortex for 5 min, centrifuge at 4000 r/min at room temperature (20-25 ℃) for 10 min.
2. Take 3 mL of clear supernatant, add 2 mL 0.1 M NaOH and 6 mL ethyl acetate, shake for 5 min, centrifuge at 4000 r/min at room temperature (20-25 ℃) for 10 min. Take supernatant (almost is clear), blow to dry with nitrogen or air at 50 ℃.
3. Add 1 mL tri-deionized water(tri-deionized water should be distilled 3 times for use), redissolve residues properly.
4. Take 20 µL for analysis.

Fold of dilution of sample: 1

5.3 *Feed*

1. Grind sample, weigh 2.0±0.05 g, add 2 mL 1 M HCI and 16 mL deionized water. then homogenize it .
2. Vortex for 3 min, then shake with oscillator for 5 min.
3. Centrifuge at above 4000 r/min for 15 min, take supernatant (must be clear), add 2 mL 1 M NaOH, mix evenly, adjust pH to 6-8.
4. Centrifuge at above 4000 r/min for 15 min, take supernatant (must be clear).
5. Diluted with tri-deionized water at 1:9 (100 µL clear upper liquid + 900 µL tri-deionized water).
6. Take 20 µL for analysis.

Fold of dilution of sample: 100

6. Sample storage

1. Untreated samples are stored at frozen environment.
2. HCI - acidified homogenates are stable at 2-8 ℃for up to 3 days
3. Prepared sample can be stable at 2-8 ℃ for 1 week.

7. 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. Numbering: number the micro-wells according to samples and standard solution; each sample and standard solution should be performed in duplicate; record their positions.
3. Add 20 μL of the sample or the standard solution into separate duplicate wells, then add enzyme conjugate, 50 μL/well; and antibody working solution, 80 μL/well. Mix gently by shaking the plate manually, seal the microplate with the cover membrane, incubate at 25 ℃for 30 min.
4. Pour liquid out of microwell, flap to dry on absorbent paper; add 250 μL/well of tri-deionized water, wash for 4-5 times, 15-30 s each time, then take out and flap to dry with absorbent paper.
5. 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 25 ℃for 15 min in the dark for coloration.
6. Determination: add 50 μL of the 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 β-agonists concentration in the sample.

8.1 Qualitative determination

The concentration range (ng/mL) 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 0.1 ppb, 1.175 for 0.30 ppb, 0.751 for 1ppb, 0.421 for 5 ppb ,0.198 for 10ppb, accordingly the concentration range of the sample Ⅰ is 5 to 10 ppb, and that of the sample Ⅱ is 0.30 to 1 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

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

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$\frac{B}{B_0}$

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

B_0—the average OD value of the 0 ng/mL standard solution

Draw the standard curve with the absorption percentages of standard solutions and the semilogarithm values of β-agonists 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 dilution fold, finally obtaining β-agonists concentration in the sample.

Using the professional software of this kit will be more convenient for accurate and rapid analysis of a large amount of samples.

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.
12. The optimum reaction temperature is 25 ℃, and too high or too low temperatures will result in the changes in the detecting sensitivity and OD values.

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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This kit is only used in research or experiment, not used in human or animal diagnosis.

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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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