VERSION 1.01

CLENBUTEROL ELISA TEST KIT

MANUAL

ELISAKITS.ONLINE

By Immunomart

Clenbuterol ELISA Test Kit

Catalogue Number. IP100038

Principle

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

Technical specifications

Sensitivity: 0.1 ppb
Incubation Temperature: 25℃
Incubation Time: 30min—15min
Detection limit: Urine 0.1 ppb, Tissue (method one) 0.4 ppb, Tissue (method two) 0.1 ppb, Feed 1 ppb
Recovery rate: Urine 95±22%, Tissue 75±20%, Feed 80±20%
Cross-reaction rate: Clenbuterol 100%, Terbutalin<1%, Mabuterol<1%, Brombuterol<1%, Salbutamal < 1%
Ractopamine < 1%

Components

1	Micro-well strips	12 strips with 8 removable wells each	
2	6× standard solution (1mL each)	0 ppb, 0.1 ppb, 0.3 ppb, 0.9 ppb, 2.7 ppb ,8.1 ppb	
3	Enzyme conjugate	7ml	red cap
4	Antibody working solution	10ml	blue cap
5	Substrate A	7ml	white cap
6	Substrate B	7ml	Black cap
7	Stop solution	7ml	yellow cap

Materials required but not provided

Equipments: microplate reader, printer, homogenizer, nitrogen-drying device, vortex, oscillator, centrifuge, measuring pipets, balance (a sensibility reciprocal of 0.01 g)

Micropipettors: single-channel 20-200 µL, 100-1000 µL, and multi-channel 30～300 µl;

Reagents: Acetonitrile (CH_3CN), NaOH, ethyl acetate, methanol, n-Hexane, HCl (approx 36.5%), Na_2SO_4(100%)

Sample pre-treatment

Instructions

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) 0.1 M HCl: dissolve 0.86 mL HCl (approx 36.5%) in deionized water to 100 mL.
2) 0.1 M NaOH: dissolve 0.4 g NaOH in deionized water to 100 mL.
3) CH_3CN-0.1 M HCl solution: $V_{CH3CN} : V_{HCl}$ =84:16

Samples preparation

a) *Urine*

Take 20 µL clear urine, directly detect it (If urine is muddy, must filter or centrifuge at 4000 r/min for 10 min, then take clear urine). Store at frozen environment if don't use.
Fold of dilution of sample: 1

b) *Tissue*

Method one

1. Weigh 2±0.05 g homogeneous sample, put it into a 50ml centrifuge tube, add 6 ml deionized water, shake thoroughly for 2 min, centrifuge at above 4000 r/min at room temperature (20-25 ℃) for 10 min.(Tips: If the fat content is higher in the sample, can firstly put it in the water bath at 85 ℃ for 10 min after shaking, then centrifuge)
2. Take 20 µL clear supernatant for analysis.
Fold of dilution of sample: 4

Method two

1. Weigh 2±0.05 g homogeneous sample, put it into a 50ml centrifuge tube, add 6 mL CH_3CN-0.1 M HCl solution, shake for 2 min, centrifuge at above 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 1 min, centrifuge at above 4000 r/min at room temperature (20-25 ℃) for 10 min. Take all supernatant (almost is clear), blow to dry with nitrogen or air at 50～60 ℃.
3. Add 1 mL deionized water, mix for 30s, redissolve residues properly.
4. Take 20 µL for analysis.
Fold of dilution of sample: 1

c) *Feed*

1. Grind sample, weigh 1.0±0.05 g, put it into a 50ml centrifuge tube, add 10ml methanol, then add 5g Na_2SO_4, shake thoroughly for 2 min, centrifuge at above 4000 r/min at 15℃ for 10 min
2. Take 1 ml supernatant (must be clear), blow to dry with nitrogen or air at 50～60℃. Add 1 ml deionized water to dissolve the dry residue, then add 1 mL N-hexane. Mix properly for 30 seconds, centrifuge at above 4000 r/min at room temperature 15 ℃ for 5 min. Remove up-layer organic phase.
3. Take down-layer phase 20 µL for analysis.
Fold of dilution of sample: 10

ELISA procedures

Instructions

1. Bring all reagents and micro-well strips to the room temperature (20-25℃).
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.

Operation 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 deionized water, wash for 4-5 times, 15-30 s each time, then take out and flap to dry with absorbent paper (if there are the bubbles after flapping, cut them with the clean tips).
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).

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 Clenbuterol concentration in the sample.

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 0.3, and that of the sample Ⅱ is 1.0, the OD value of standard solutions is: 2.243 for 0 ppb, 1.816 for 0.1 ppb, 1.415 for 0.30 ppb, 0.74 for 0.90 ppb, 0.313 for 2.7 ppb, 0.155 for 8.1ppb, accordingly the concentration range of the sample Ⅰ is 2.7 to 8.1 ppb, and that of the sample Ⅱ is 0.30 to 0.90 ppb

Quantitative determination

The mean values of the absorbance values obtained for the percentage of 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,

Percentage of absorbance value = $(B / B_0) \times 100\%$

B—the average 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 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 dilution fold, finally obtaining clenbuterol 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.

Precautions

1. The room temperature below 25 ℃ 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.
2. 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.
3. Mix evenly, otherwise there will be the undesirable reproducibility.
4. The stop solution is the 2 M sulfuric acid solution, avoid contacting with the skin.
5. 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.
6. 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.
7. 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.
8. 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.

Storage: store at 2-8 ℃, not frozen.
Expiry date: 12 months; date of production is on box.