Elabscience®

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Clenbuterol ELISA Kit

Catalog No: E-FS-E014

96T

This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help.

Phone: 240-252-7368(USA)240-252-7376(USA)
Email: techsupport@elabscience.com
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Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Test principle

This kit uses Indirect-Competitive-ELISA as the method. It can detect Clenbuterol (CLE) in urine, tissues and feed. This kit is composed of Micro ELISA Plate pre-coated with coupled antigen, HRP conjugate, antibody, standard and other supplementary reagents. During the detection, after adding standard or sample solution, CLE in the samples competes with pre-coated coupled antigen on the Micro ELISA Plate for CLEantibody.Then Horseradish Peroxidase (HRP) conjugate is added to each micro plate well, and TMB substrate is added for color development. There is a negative correlation between the OD value of samples and the concentration of CLE. The residual quantity of CLE in the samples can be calculated by comparing the OD of the samples to the standard curve.

Technical indicator

Sensitivity: 0.1ppb (ng/mL)

Reaction mode: 25℃, 30min~15min

Detection limit: Urine ---0.1ppb; Tissue (treatment method 1) ---0.4ppb; Tissue (treatment method 2) ---0.1ppb; Feed---1ppb.

Cross-reactivity: Clenbuterol ---100%; Arubendol---<1%; Mabuterol---<1%;Brombuterol---<1%; Albuterol---<1%;Ractopamine---<1%.

Sample recovery rate: Urine ---95%±10%; Tissue, Feed---85%±15%.

Kits components

Item	Specifications
Micro ELISA Plate	96 wells
Standard Liquid	1mLeach. 0ppb,0.1ppb,0.3 ppb,0.9 ppb, 2.7ppb, 8.1ppb.
High Concentration Standard (Red cap)	100 ppb, 1 mL
HRP conjugate (Red cap)	5.5mL
Antibody Working Solution(Blue cap)	9mL
Substrate Reagent A(White cap)	6mL
Substrate Reagent B(Black cap)	6mL
Stop Solution(Yellow cap)	6mL
20×Concentrated Wash Buffer (White cap)	40mL
10×Redissolved Buffer (Yellow cap)	50 mL
Product Description	1 copy

Other supplies required

Instruments: Micro-plate reader, Printer, Homogenizer, Nitrogen Evaporators, Oscillators, Centrifuge, Graduated pipette, Balance (sensibility 0.01g).

High-precision transferpettor: Single channel (20-200μL, 100-1000μL), Multichannel (300μL).

Reagents: NaOH,Acetic ether,Concentrated HCl, Acetonitrile, Methanol, N-hexane, Anhydrous sodium sulfate.

Experimental preparation

1. **Sample pretreatment Notice:**Experimental apparatus should be clean and the pipette should be disposable to avoid cross-contamination during the experiment.

2. **Reagent preparation**

Reagent 1: 0.1MHCl solution

Dilute 0.86 mL Concentrated HCl with deionized water to 100 mL.

Reagent 2:0.1M NaOH solution

Dissolve 0.4 g NaOH with 100mL deionized water.

Reagent 3: Acetonitrile-0.1M HCl solution

Volume (Acetonitrile): Volume (0.1M HCl solution) =84:16.

Reagent 4: Recover-solution

Dilute the 10×Recover solution with deionized water for re-dissolution of samples. The recover-solution can be stable for 1 month at 4℃.

3. **Sample pretreatmentprocedure**

3.1 Pretreatmentof urine sample

Take 20 μL clear urine sample for analysis directly ((if the urine sample is muddy, it should be filtered or centrifuged at 4000 r/min for 5 min until the urine sample become clear). Samples temporarily not used should be frozen.

Note:Sample dilution factor: 1, minimum detection dose: 0.1ppb.

3.2 Pretreatment oftissue (pretreatment method 1)

Weigh 2±0.05g ofcrushedhomogenate tissue sample, add6mLrecover-solution. Oscillate fully for 2 min, centrifuge at a speed of over 4000r/min for 10min (incubate the sample at 85℃ for 10 min before centrifugation if there is a high-content of fat in tissue sample).Take 20 μLof the supernatant for analysis.

Note:Sample dilution factor: 4, minimum detection dose: 0.4ppb.

3.3 Pretreatment of tissue (pretreatment method 2)

(1) Weigh 2±0.05g of crushedhomogenate tissue sample, add 6 mL of acetonitrile-0.1M HCl solution. Oscillate for 2 min, centrifuge at a speed of over 4000r/min for 10min at room temperature.

(2) Take 3mLof the supernatant. Add 2mL of 0.1M NaOH and 6 mL acetic ether. Oscillate fullyfor 2

min, centrifuge at a speed of over 4000r/min for 10min at room temperature. Take all the supernatant and dry with nitrogen or airat 50-60℃.

(3) Add 1 mL recover-solution and oscillate for 30 seconds. Take 20 μL for analysis.
Note: Sample dilution factor: 1, minimum detection dose: 0.1ppb.

3.4 Pretreatment of feed sample

(1) Weigh 1±0.05 g of homogenate feed sample, add 10mLmethanol and 5 g Na_2SO_4.Oscillate for 2 min, centrifuge at a speed of over 4000r/min for 10min at room temperature.

(2) Take 1 mL of the supernatant and dry with nitrogen or airat 50-60℃.Add 1 mLof recover-solution to dissolve the remaining dry material. Then add 1 mL n-hexane and mix for 30 seconds.Centrifuge for 5 min at a speed of over 4000 r/min at room temperature.

(3) Take 20 μLof the lower layer liquid for analysis.
Note: Sample dilution factor: 10, minimum detection dose: 1 ppb.

Assay procedure

Bring all reagents and samples to room temperature for more than 30 min before use. The washing solution may be crystallized when refrigerated, therefore it should be adjusted to room temperature to fully dissolve. Each liquid must be shaken up before use. Take out the Micro plate and frame of needed amount, and keep the remained Micro plate into the valve bag and stored at 2-8℃.

Dilute the 20×concentrated wash buffer to wash working buffer before the experiment.

1. **Number:** number the sample and standard in order (multiple well), and keep a record of standard wells and sample wells.
2. **Add sample:**add 20μL of Standard, Blank, or Sample per well, then add 50 μLHRP conjugate to each well. Add 80 μLantibody working solution, cover the plate with sealer we provided, oscillate for 5s gently to mix thoroughly, shading light incubation for 30min at25℃.
3. **Wash:** uncover the sealer carefully, remove the liquid in each well. Immediately add 250μLof wash working buffer to each well and wash.Repeat wash procedure for 5 times, 30s intervals/time. Invert the plate and pat it against thick clean absorbent paper (If bubbles exist in the wells, clean tips can be used to prick them).
4. **HRP conjugate:**add 100μL HRP conjugate to each well, shading light incubation for 30min at 25℃.
5. **Wash:** repeat step 3
6. **Color Development:** add 50μLof substrate solution A to each well, and then add 50μLof substrate solution B.Gently oscillate for 5s to mix thoroughly. Incubate shading light for 15min at 25℃.
7. **Stop reaction:** add 50μLof stop solution to each well,gently oscillate and mix fully to stop the reaction.
8. **OD Measurement:** determine the optical density (OD value) of each wellat 450 nm with a microplate reader (the 450/630 nm double wavelength is recommended). This step should be finished in 10min after stop reaction.

Result analysis

1. **Absorbance% =A/A_0×100%**

   A: Average absorbance of standard solution or sample

   A_0: Average absorbance of 0 ppb Standard solution

2. **Drawing and calculation of standard curve**

   Create a standard curve by plotting the absorbance percentage of each standard on the y-axis against the log concentration on the x-axis to draw a semi-logarithmic plot. Add the average absorbance value of sample to standard curve to get corresponding concentration. If samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor.

   For this kit, it is more convenient to use professional analysis software for accurate and fast analysis on many samples.

Notes

1. Overall OD value will be lower when reagents have notbeen brought to room temperature before use or room temperature is below25℃.
2. During the washing procedure, if the wells turn dry, it will lead to bad linear standard curve andpoor repeatability, move on to the next step immediately after wash.
3. Mix thoroughly and wash the plate completely. The consistency of wash procedure can strongly affect the reproducibility of this ELISA kit.
4. Micro ELISA plate should be covered by plate sealer. Avoid the reagents to strong light.
5. Do not use expired kit and reagents of different batches.
6. TMB should be abandoned if it turns color. When OD value of standard(concentration: 0)＜0.5 unit(A_{450nm}＜0.5), it indicates the reagent may be deteriorated.
7. Stop solution is caustic, avoid contact with skin and eyes.

Storage and valid period

Storage: Store at 2-8℃. Avoid freeze / thaw cycles.

Valid Period: 1 year, production date is on the packing box.