Elabscience®

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CIM (Cimaterol) ELISA Kit

Catalog No: E-FS-E026

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
Website: www.elabscience.com

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 CIM (Cimaterol) 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, CIM in the samples competes with pre-coated coupled antigen on the Micro ELISA Plate for CIM antibody. 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 CIM. The residual quantity of CIM in the samples can be calculated by comparing the OD of the samples to the standard curve.

Technical indicator

Sensitivity: 0.3 ppb (ng/mL)

Reaction mode: 25℃, 30 min~15 min

Detection limit: Urine ---0.3 ppb, Tissue (treatment method 1) ---1.2 ppb,
Tissue (treatment method 2) ---0.3 ppb, Feed---3 ppb.

Cross-reactivity: Clenbuterol ---100%, Arubendol---<1%, Mabuterol---<1%, Brombuterol---<1%, Salbutamol ---<1%, Ractopamine---<1%.

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

Kits components

Item	Specifications
Micro ELISA Plate	96 wells
Standard Liquid (black cap)	1mL each (0 ppb, 0.3 ppb, 0.6 ppb, 1.2 ppb, 2.4 ppb, 4.8 ppb)
High Concentrated Standard (100 ppb)	1 mL
HRP Conjugate (red cap)	5.5 mL
Antibody Working Solution(blue cap)	5.5 mL
Substrate Reagent A(white cap)	6 mL
Substrate Reagent B(black cap)	6 mL
Stop Solution(yellow cap)	6 mL
20×Concentrated Wash Buffer (white cap)	40 mL
10×Reconstitution Buffer (yellow cap)	50 mL
Plate Sealer	3 pieces
Sealed Bag	1 piece
Manual	1 copy

Other supplies required

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

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

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: 0.1 M HCl Solution

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

Reagent 2: 0.1 M NaOH Solution

Dissolve 0.4 g NaOH with 100 mL deionized water.

Reagent 3: Acetonitrile-0.1 M HCl Solution

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

Reagent 4: Reconstitution Solution

Dilute the 10×Reconstitution Buffer with deionized water. (10×Reconstitution Buffer :deionized water=1:9) .The Reconstitution solution can be store at 4℃ for a month.

Reagent 5: Wash Buffer

Dilute 20×Concentrated Wash Buffer with deionized water. (20×Concentrated Wash Buffer (V): Deionized water (V) = 1:19)

3. Sample pretreatment procedure

3.1 Pretreatment of urine sample:

Take 20 μL of 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: 0.3 ppb.

3.2 Pretreatment of tissue (pretreatment method 1):

Weigh 2± 0.05 g of crushed homogenate tissue sample, add 6 mL of Reconstitution solution. Oscillate fully for 2 min, centrifuge at a speed of over 4000 r/min for 10 min (incubate the sample at 85℃ for 10 min before centrifugation if there is a high-content of fat in tissue sample). Take 20 μL of the supernatant for analysis.

Note: Sample dilution factor: 4, minimum detection dose: 1.2 ppb.

3.3 Pretreatment of tissue (pretreatment method 2):

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

(2) Take 3 mL of the supernatant. Add 2 mL of 0.1 M NaOH and 6 mL of acetic ether. Oscillate fully for 2 min, centrifuge at a speed of over 4000 r/min for 10 min at room temperature. Take all the supernatant and dry at 50-60℃with nitrogen or air.

(3) Add 1 mL of Reconstitution Solution and oscillate for 30 sec. Take 20 μL for analysis.
Note: Sample dilution factor: 1, minimum detection dose: 0.3 ppb.

3.4 Pretreatment of feed sample:

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

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

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

Assay procedure

Centrifuge the sample again after thawing before the assay. Bring all reagents to room temperature before use. All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming.

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 50 μL of Standard, Blank, or Sample per well, then add 50 μL HRP Conjugate to each well. Add 50 μL of antibody working solution, cover the plate with sealer we provided, oscillate for 5 sec gently to mix thoroughly, shading light incubation for 30 min at 25℃.
3. **Wash:** uncover the sealer carefully, remove the liquid in each well. Immediately add 250μL of wash buffer to each well and wash. Repeat wash procedure for 5 times, 30 sec 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. **Color Development:** add 50 μL of substrate solution A to each well, and then add 50 μL of substrate solution B. Gently oscillate for 5 sec to mix thoroughly. Incubate shading light for 15 min at 25℃.
5. **Stop reaction:** add 50 μL of stop solution to each well, gently oscillate and mix fully to stop the reaction.
6. **OD Measurement:** determine the optical density (OD value) of each well at 450 nm with a micro-plate reader (the 450/630 nm double wavelength is recommended). This step should be finished in 10 min 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 not been brought to room temperature before use or room temperature is below 25℃.
2. During the washing procedure, if the wells turn dry, it will lead to bad linear standard curve and poor 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($A_{450\ nm}$＜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.