Cimaterol (CIM) ELISA Kit

Technical Manual

(ELISA)

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1 Principle and Application

This kit adopts the method of indirect competitive enzyme-linked immunoassay (ELISA) to detect Cimaterol (CIM) in the sample such as urine, tissue, feed. The kit is composed of Microtiter Plate coated with coupled antigens, HRP conjugate, antibodies, standards and other supporting reagents. During the detection, with adding standards or samples, the CIM in the samples will compete with the coupled antigens to combine with anti-CIM antibodies. After adding HRP conjugates, take coloration with TMB substrates. Absorbance value of the samples is a negative correlation with CIM content. Lastly, comparing with the standard curve, the obtained concentration is multiplied by the sample dilution ratio. CIM residues in sample can be concluded.

2 Technique Data

2.1 Kit Sensitivity: 0.3ppb (ng/mL)

2.2 Reactive Mode: 25°C, 30min ~ 15min

2.3 Detection Limits:

Sample	Detection Limits
Urine	0.3ppb
Tissue (Treatment 1)	1.2ppb
Tissue (Treatment 2)	0.3ppb
Feed	3ppb

2.4 Cross-reaction Rate:

Cimaterol 100%
Terbutalin ..... <1%
Mabuterol ..... <1%
Brombuterol ..... <1%
Salbutamol ..... <1%
Ractopamine ..... <1%

2.5 Sample Recovery Rate:

Sample	Recovery rate
Urine	95±15%
Tissue, feed	85±15%

3 Composition of the Kit

Reagent	Specification
Microtiter Plate	8wells× 12strips
Standard: 0ppb, 0.1ppb, 0.3ppb, 0.9ppb, 2.7ppb, 8.1ppb (black cap)	1.0mL each
High Standard (black cap): 100ppb	1×1.0mL
Antibody solution (blue cap)	1×5.5mL
HRP conjugate (red cap)	1×5.5mL
Substrate Reagent A (white cap)	1×6mL
Substrate Reagent B (black cap)	1×6mL
Stop Solution (yellow cap)	1×6mL
Concentrated Wash Buffer (20×)(white cap)	1×40mL
Concentrated Reconstitution Buffer (10×) (yellow cap)	1×50mL
Instruction	1
Adhesive Membrane	1
Sealed bag	1

4 Materials Required but Not Supplied

4.1 Equipment: microplate reader, printer, grinder (for homogenizing solid samples), nitrogen evaporator, vortex mixer (for shake and mix), centrifuge, graduated transfer pipette, and balance with a division value of 0.01 g, constant temperature device, water bath;

4.2 Micropipette: single-channel (20-200μL and 100-1000μL), and multi-channel 300μL;

4.3 Reagents: Sodium Hydroxide (NaOH), Ethyl Acetate, Concentrated Hydrochloric Acid (36% HCl), Acetonitrile, Methanol, Anhydrous Sodium Sulfate, n-hexane.

5 Experimental preparation

Restore all reagents and samples to room temperature (adjust to around 25°C) for more than 30 min before use. This is a crucial step to ensure there is no precipitation in the reagents.

5.1 Notice Before Sample Processing:

Please note that the labware must be clean. Use disposable pipette tips to avoid contamination of interference results.

5.2 Solution preparation:

Solution 1: 0.1M Hydrochloric Acid

Mix 0.86 mL of concentrated hydrochloric acid with deionized water and dilute to a total volume of 100 mL.

Solution 2: 0.1M Sodium Hydroxide

Dissolve 0.4 g of sodium hydroxide in deionized water

and dilute to a total volume of 100 mL.

Solution 3: Acetonitrile-0.1M Hydrochloric Acid Mixture

Mix acetonitrile and 0.1M hydrochloric acid in a volume ratio of 84:16.

Solution 4: Reconstitution Buffer

Dilute the Concentrated Reconstitution Buffer (10×) 10 times with deionized water (Reconstitution Buffer (10×): deionized water=1:9). The solution can be stored at 4 °C for one month.

Solution 5: Working Wash Buffer

Dilute the concentrated wash buffer (20×) by a factor of 20 (Concentrated wash buffer/Deionized water= 1: 19).

5.3 Sample pretreatment steps:

5.3.1 Urine treatment.

1) Directly take 50 µL of clear urine sample for measurement (cloudy urine samples need to be filtered or centrifuged at 4000 r/min for 5 minutes to obtain a clear sample). Samples not in use should be stored frozen.

Dilution times of the sample:1 Detection limits: 0.3ppb

5.3.2 Tissue treatment 1.

1) Weigh 1g ± 0.05g of homogenized tissue sample and add 3mL of reconstitution buffer (Solution 4). Vigorously shake for 2 minutes. Centrifuge at room temperature at 4000 r/min for 10 minutes. (If the tissue sample has a high fat content, place it in an 85°C water bath for 10 minutes after shaking, then centrifuge.).

2) Take 50µL of the supernatant for analysis.

Dilution times of the sample:4 Detection limits: 1.2ppb

5.3.3 Tissue treatment 2.

1) Weigh 2g ± 0.05g of homogenized tissue sample and add 6 mL of acetonitrile-0.1M hydrochloric acid solution (Solution 3). Vigorously shake for 2 minutes, then centrifuge at room temperature at 4000 r/min for 10 minutes.

2) Take 3 mL of the supernatant, add 2 mL of 0.1M sodium hydroxide solution (Solution 2), and then add 6 mL of ethyl acetate. Vigorously shake for 2 minutes, centrifuge at room temperature at 4000 r/min for 10 minutes, and evaporate 3 mL of the supernatant under nitrogen or air at 50-60°C until dry.

3) Add 0.5 mL of reconstitution buffer (Solution 4) and mix thoroughly for 30 seconds. Take 50 µL for analysis.

Dilution times of the sample:1 Detection limits: 0.3ppb

5.3.4 Feed treatment.

1) Weigh 1g ± 0.05g of homogenized feed sample, add 10 mL of methanol, then add 5g of anhydrous sodium sulfate. Shake for 2 minutes, then centrifuge at room temperature at 4000 r/min for 10 minutes.

2) Extract 1 mL of the supernatant and evaporate it to dryness under nitrogen or air at 50-60°C. Dissolve the dried residue with 1 mL of reconstitution buffer (Solution 4), then add 1 mL of n-hexane and mix for 30 seconds. Centrifuge at room temperature at 4000 r/min for 5 minutes.

3) Take 50 µL of the lower layer for analysis.

Dilution times of the sample:10 Detection limits: 3ppb

6 ELISA procedure

Place all reagents and samples to room temperature (adjust to around 25°C) for 30min. Gently shake the reagent bottles before use.

Take out the frame of the microplate along with the required number of wells. Then place the unused microplate wells into the sealed bag with the desiccant provided. Store the remaining kit in the refrigerator at 2-8°C.

Step 1: Number: Number the samples and standard corresponding microwells in order, make 2-well parallel trials for each sample and standard, and record the locations of sample wells and standard wells.

Step 2: Incubation: Add 50µL of standard or sample into each numbered well, then add 50µL of HRP conjugate per well. Next, add 50µL of antibody solution into each well. Finally, cover the Microtiter Plate with the adhesive membrane, shake gently by hand (or use a microplate shaker) for 5s and incubate for 30 min at 25°C.

Step 3: Washing: Uncover the adhesive membrane carefully, discard liquid in the wells, pipette 350µL of Working Wash Buffer (Solution 5) to every well, let stand for 30 seconds then drain, repeat 5 times. Invert the plate and tap it against a thick absorbent paper (or lint-free cloth), with a soft towel placed underneath. (Bubbles that are not removed after patting dry can be punctured with a clean pipette tip).

Step 4: Color: Add 50µL of Substrate Reagent A to each well. Then add 50µL of Substrate Reagent B per well. Shake gently by hand (or use a microplate shaker) for 5s, and allow to react for 15min at 25°C in the dark. (The reaction can be extended appropriately if the blue color is too pale.)

Step 5: Stop the reaction: Pipette 50µL of Stop Solution to each well, and shake gently by hand (or use a microplate shaker). The reaction would be stopped.

Step 6: Calculate: Determine the Optical Density (OD value; absorbance value) at 450nm (Reference wavelength 630nm) with a microplate reader. Finish this step within 10min after stop the reaction.

7 Interpretation of result

7.1 Calculate the percentage of absorbance value

Percentage of absorbance value(%)= $\frac{A}{A0}$ ×100%

A—the average OD value of the sample or standard;

A0—the average OD value of the 0ppb standard.

It is used to calculate the percentage absorbance of a standard or sample.

7.2 Draw the standard curve and calculate

Take absorbance percentage (A/A0) of standards as Y-axis and the corresponding log of standards concentration (ppb) as X-axis.

Draw the standard semi-log curves with X-axis and Y-axis.

Take absorbance percentage of samples substitute into standard curve, then can get the corresponding concentration from standard curve. **Last, the resulting concentration values multiplied by the corresponding dilution times is the actual concentration of CIM of samples.**

If professional analysis software of the kit is used for calculation, it is more convenient for accurate and rapid analysis of a large number of samples.

8 Attention

8.1 Before test, the reagents and samples should be balanced to room temperature (25°C). If below 25°C, it will lead to all the standard OD value on the low side.

8.2 In the washing process, dry wells may result in non-linear standard curves and undesirable reproducibility. Therefore, proceed to the next step immediately after washing.

8.3 Please mix the contents within the wells uniformly and wash the plate thoroughly. The reproducibility is largely determined by consistency of washing step.

8.4 During the incubation, cover microplates with adhesive membrane to avoid light.

8.5 Do not use kits that are overdue. Do not mix reagents with those from other lots.

8.6 Substrate Reagent A/B is colorless. If not, please discard.

8.7 If absorbance value of 0ppb is below 0.5（A450nm< 0.5), it means that the reagent may be metamorphic.

8.8 Stop solution is corrosives, please avoid contact with skin.

8.9 As the OD values of the standard curve may vary according to the conditions of actual assay performance (e.g. operator, pipetting technique, washing technique or temperature effects), the operator should establish a standard curve for each test.

8.10 For the mentioned sample, fast and efficient extraction methods are included in the kit description. Please consult technical support for the applicability if other sample need to be tested.

8.11 The kit is used for rapid screening of actual samples. If the test result is positive, the instrument method such as HPLC, LC/MS can be used for quantitative confirmation.

9 Storage conditions

The kit shall be stored at 2-8 °C. Avoid freezing.

Shelf Life: 12 months. The date of manufacture is presented in the label of the box.