QUALICHEK™ Cimaterol ELISA

REF : KBFP1010

Ver 1.0

RUO

Quantitative testing of Cimaterol in samples, such as urine, tissues and feed, etc.

RUO	**For Research Use Only**	**REF**	**Catalog Number**
	**Store At**	**LOT**	**Batch Code**
	**Manufactured By**		**Biological Risk**
	**Expiry Date**		**Consult Operating Instructions**

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Introduction:

Cimaterol is a chemically stable nonselective agonist β1-, β2-, and β3-adrenoceptors. Cimaterol Increases lean yield, i.e., muscle mass, while also increasing feed efficiency would greatly benefit the beef cattle industry.

Intended Use:

This kit is for quantitative testing for Cimaterol in sample, such as urine, tissues and feed etc.

Principle:

This kit uses Indirect-Competitive-ELISA as the method. It can detect CIM in samples, such as urine, tissues and feed etc. This kit is composed of ELISA Microtiter plate, HRP conjugate, antibody, standard and other supplementary reagents. The microplate provided in this kit has been pre-coated with coupled antigen. During the reaction, CIM in the samples or standard competes with coupled antigen on the solid phase supporter for sites of CIM antibody. Then Horseradish Peroxidase (HRP) conjugate is added to each micro plate well, and TMB substrate is for color development. There is a negative correlation between the OD value of samples and the concentration of CIM. The concentration of CIM in the samples can be calculated by comparing the OD of the samples to the standard curve.

Materials Provided:

1. Coated Microtiter plate - 1x 96 wells
2. Standards Liquid -1mL each (0 ppb, 0.3 ppb, 0.6 ppb, 1.2 ppb, 2.4 ppb, 4.8 ppb)
3. High Concentrated Standard (100 ppb) - 1mL
4. HRP Conjugate - 5.5 mL
5. Antibody Working Solution - 5.5 mL
6. Substrate A - 6 mL
7. Substrate B - 6 mL
8. Stop Solution - 6 mL
9. Wash Buffer (20X) - 40 mL
10. Plate Sealer - 3 pieces

Material required but not provided:

Instrument: Microtiter plate reader, Printer, Homogenizer, Nitrogen Evaporators/Water bath, Oscillators, centrifuge, Graduated pipette, Balance (sensibility 0.01 g)
High-precision transferpettor:single channel (20-200 µL, 100-1000 µL), Multichannel (300 µL) micropipettes.
Reagents: NaOH, Acetic ether, Concentrated HCl, Acetonitrile, Methanol, N-hexane, Anhydrous sodium sulfate (Na_2SO_4).

Storage Information:

1. Store main kit components at 2-8°C Avoid freeze / thaw cycles.
2. Before using, bring all components to room temperature (18-25°C). Upon assay completion return all components to appropriate storage conditions.

Sample pretreatment:

Bring all reagents and samples to room temperature before use.

Open the microplate reader in advance, preheat the instrument, and set the testing parameters.

1. Sample pretreatment Notice:

Experimental apparatus should be clean, and the pipette should be disposable to avoid cross-contamination during the experiment.

2. Solution preparation:

Solution 1: 0.1 M HCl Solution: Dilute 0.86 mL Concentrated HCl with deionized water to 100 mL.

Solution 2: 0.1 M NaOH Solution: Dissolve 0.4 g NaOH with 100 mL deionized water.

Solution 3 Acetonitrile-0.1 M HCl Solution: Volume (Acetonitrile): Volume (0.1 M HCl solution) =84:16.

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

Solution 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:

Pretreatment of urine sample:
Take 50 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.

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 50
µL of the supernatant for analysis.
Note: Sample dilution factor: 4, minimum detection dose: 1.2 ppb.

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 roomtemperature.
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 buffer and oscillate for 30 sec. Take 50 µL for analysis.

Note: Sample dilution factor: 1, minimum detection dose: 0.3 ppb.

Pretreatment of feed sample:
1. Weigh 1±0.05 g of homogenate feed sample, add 10 mL of methanol and 5 g of Na2SO4. 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 evaporators/water bathat 50-60℃. Add 1 mL of Reconstitution buffer 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 50 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 for 30 min 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 or Sample per well, then add 50 µL of HRP conjugate to each well, then add 50 µL of antibody working solution, cover the plate with sealer, oscillate for 5 s gently to mix thoroughly, incubate for 30min at 25℃.
3. **Wash:** uncover the sealer carefully; remove the liquid in each well. Immediately add 300 µL of wash buffer to each well and wash. Repeat wash procedure for 5 times, 30 s 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 Reagent A to each well, and then add 50 µL of Substrate Reagent B. Gently oscillate for 5 s to mix thoroughly. Incubate shading light for 15 min at 25℃ (The reaction time may be shortened or prolonged according to the depth of the color).
5. **Stop reaction:** add 50 µL of stop solution to each well, oscillate gently to mix thoroughly.
6. **OD Measurement:** determine the optical density (OD value) of each well at 450 nm with a microplate reader (the 450/630 nm double wavelength is recommended). This step should be finished in 10 min after stop reaction.

Judgment of result:

Absorbance (%) =A/A0×100%

A: Average absorbance of standard or sample

A_0: Average absorbance of 0 ppb Standard

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 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 a large number of samples.

Notes:

1. The overall OD value will be lower when reagents have not been brought to room temperature before use or room temperature is below 25℃.
2. If the wells turn dry during the washing procedure, it will lead to bad linear standard curve and poor repeatability. Operate 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. ELISA Microtiter plate should be covered by plate sealer. Avoid the reagents to strong light.
5. Do not use expired kit or reagents of different batches.
6. TMB should be abandoned if it turns color. When OD value of standard (concentration: 0)＜0.5 unit (A450nm＜0.5), it indicates the reagent may be deteriorated.
7. Stop solution is caustic; avoid contact with skin and eyes.

Technical specification:

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.
Reactivity: Clenbuterol ---100%, Arubendol---<1%, Mabuterol---<1%,
Brombuterol---<1%, Salbutamol ---<1%, Ractopamine---<1%.
Sample recovery rate: Urine ---95%±10%, Tissue, Feed---85%±15%.

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