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β-agonist (Beta-Agonist) ELISA Kit

Cat# **E5021**

Storage at **2-8˚C for 1 year**

SPECIFICATION

Sensitivity: 0.2 ppb (ng/mL)

Reaction mode: 25℃, 15 min~15 min ~15 min

Detection limit: Urine --- 1 ppb; Muscle --- 1 ppb

Cross-reactivity:

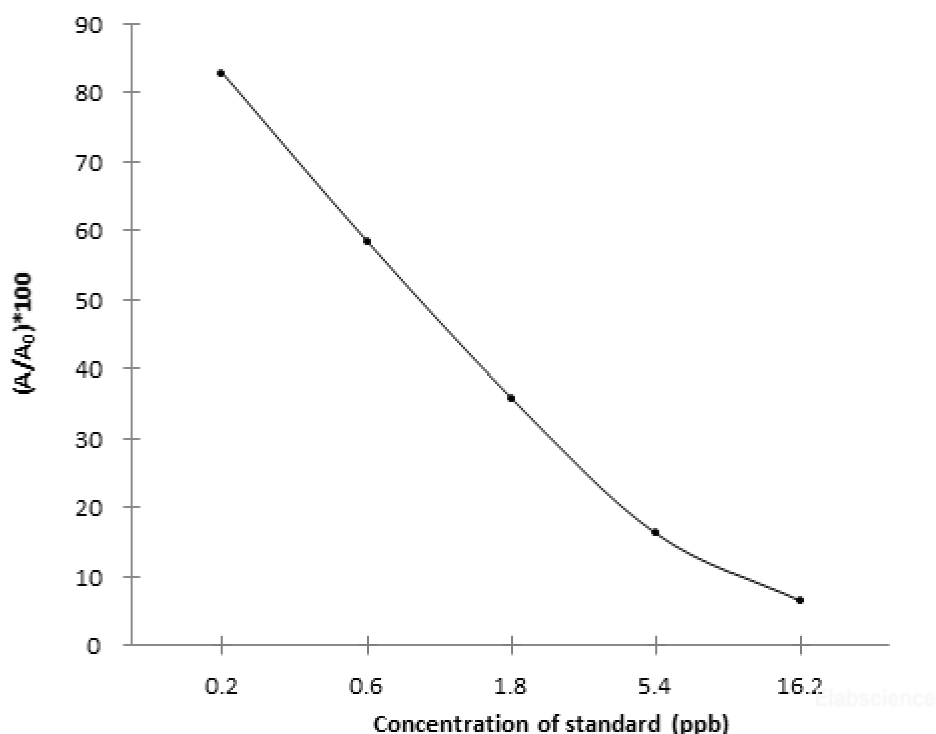
Clenbuterol	100%
Clorprenaline	141%
Tulobuterol	80%
Brombuterol, Mapenterol	107%
Cimbuterol	86.9%
Clenpenterol	78.3%
Terbutaline	60.6%
Salbutamol	60.6%
Mabuterol	56.3%
Bambuterol	18%
Cimaterol	14%
Clenproperol	5%
Ractopamine, Zilpaterol, Phenylethanolamine A	＜1%

Sample recovery rate: 90%±30%.

PRINCIPLE of KIT

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

KITS COMPONENTS



Item	Specifications
ELISA Microtiter Plate	96 wells
Standard Liquid	1 mL each (0 ppb, 0.2 ppb, 0.6 ppb, 1.8 ppb, 5.4 ppb, 16.2 ppb)
HRP Conjugate	12 mL
Antibody Working Solution	7 mL
Substrate Reagent A	6 mL
Substrate Reagent B	6 mL
Stop Solution	6 mL
Sample Stabilizer	5 mL
Sample Solution	30 mL
20×Concentrated Wash Buffer	25 mL
Plate Sealer	3 pieces
Sealed Bag	1 piece
Manual	1 copy

Note: All reagent bottle caps must be tightened to prevent evaporation and microbial pollution.

Other materials required but not supplied

Instrument: Microplate Reader, Homogenizer, Oscillators, Centrifuge, Balance (sensibility 0.01 g).

Micropipettor: Single-channel (20-200 μL, 100-1000 μL), Multi-channel (300 μL).

Reagents: Trichloroacetic acid, NaOH

EXPERIMENTAL PREPARATION

Restore 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: 1% Trichloroacetic acid Dilute 10 g of Trichloroacetic acid to 1000 mL with deionized water, mix fully.

   Solution 2: 1 M NaOH Solution. Dilute 4 g of NaOH to 100 mL with deionized water, mix fully.

   Solution 3: 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 (swine) sample:

   (1) Take 50 μL of fresh urine to detect directly (Filtrate or centrifuge for 5 min at 4000 r/min if the urine sample is turbid).

   Note: Sample dilution factor: 1, minimum detection limit: 1 ppb

   3.2 Pretreatment of urine (cattle, sheep) sample:

   (1) Take 1 mL of fresh urine into centrifuge tube (Filtrate or centrifuge for 5 min at 4000 r/min if the urine sample is turbid).

   (2) Add 40 μL of Sample Stabilizer, oscillate for 5 min.

   (3) Take 50 μL for detection.

   Note: Sample dilution factor: 1, minimum detection limit: 1 ppb

   3.3 Pretreatment of muscle (swine, cattle, sheep) sample:

   (1) Weigh 2±0.02 g of homogenate fresh sample into a 50 mL centrifuge tube;

   (2) Add 4 mL of 1% Trichloroacetic acid (Solution 1), oscillate for 5 min.

   (3) Centrifuge for 5 min at 4000 r/min;

   (4) Take 1 mL of intermediate layer solution into a new centrifuge tube; Note: Avoid taking the upper or lower solids.

   (5) Add 30 μL of 1 M NaOH Solution (Solution 2) and oscillate for 10s thoroughly;

   (6) Centrifuge for 5 min at 4000 r/min;

   (7) Take 50 μL of supernatant for detection immediately. Note: Sample dilution factor: 3, minimum detection limit: 1 ppb

   3.4 Pretreatment of serum (swine, cattle, sheep) sample:

   (1) Take 200 μL of serum into a 2 mL centrifuge tube; Note: Avoid hemolysis while taking blood. Take the supernatant for detection after Centrifuge if the serum is cloudy.

   (2) Add 200 μL of Sample Diluent and oscillate for 30 s;

   (3) Put the sample into water bath at 80℃ to incubate for 5 min;

   (4) Restore samples to room temperature, and take 50 μL for detection. Note: Sample dilution factor: 2, minimum detection limit: 1 ppb

Assay procedure

Restore all reagents and samples to room temperature (25℃) before use. All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming. The unused ELISA Microtiter plate should be sealed as soon as possible and stored at 2~8℃.

1. Number: number the sample and standard in order (multiple well), and keep a record of standard wells and sample wells. Standard and Samples need test in duplicate.
2. Add Sample: add 50 µL of Standard or Sample per well, add 50 µL of Antibody Working Solution to each well. Gently oscillate for 10s to mix thoroughly and cover the plate with plate sealer. Incubate at 25℃ for 15 min in shading light.
3. Wash: uncover the sealer carefully, remove the liquid in each well. Immediately add 260 µL of Wash Buffer (Solution 3) to each well and wash. Repeat the wash procedure for 4 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 100 µL of HRP Conjugate into each well, and incubate at 25℃ for 15 min in shading light.
5. Wash: Repeat step 3 for washing.
6. Color Development: add 50 µL of Substrate Reagent A to each well, and then add 50 µL of Substrate Reagent B. Gently oscillate for 15 s to mix thoroughly. Incubate for 15 min at 25℃ in shading light (The reaction time can be extended according to the actual color change).
7. Stop Reaction: add 50 µL of Stop Solution to each well, oscillate gently for 10s to mix thoroughly.
8. OD Measurement: determine the optical density (OD value) of each well at 450 nm (reference wavelength 630 nm) with a microplate reader. This step should be finished in 5 min after stop reaction.

RESULT ANALYSIS

1. Absorbance(%)=A/A0×100%

   A: Average absorbance of standard or sample

   A0: Average absorbance of 0 ppb Standard
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 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 form for accurate and fast analysis of batch samples.

NOTE

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 kit to strong light.

5. Each reagent is optimized for use in the E5021. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other E5021 with different lot numbers.
6. Substrate Reagent should be abandoned if it turns blue color. When OD value of standard (concentration: 0)＜0.8 unit (A450nm＜0.8), it indicates the reagent may be deteriorated.
7. Stop solution is caustic, avoid contact with skin and eyes.
8. As the OD values of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique, washing technique or temperature effects), the operator should establish a standard curve for each test.
9. Even the same operator might get different results in two separate experiments. In order to get reproducible results, the operation of every step in the assay should be controlled.
10. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
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, etc. can be used for quantitative confirmation

STORAGE AND EXPIRY DATE

Store the kit at 2~8℃. Do not freeze any test kit components.

Return any unused microwells to their original foil bag and reseal them together with the desiccant provided and further store at 2 - 8 °C.

Expiry date: expiration date is on the packing box.

PRODUCT USE LIMITATION

These products are intended for research use only.