TER (Terbutaline) ELISA Kit

Catalog No: E-FS-E091

96T/96T*3

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.

Toll-free: 1-888-852-8623 Tel: 1-832-243-6086 Fax: 1-832-243-6017

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 Competitive-ELISA as the method for the quantitative detection. It can detect Terbutaline (TER) in samples, such as muscle, serum, etc. This kit is composed of ELISA Microtiter plate, HRP conjugate, standard and other supplementary reagents. The microtiter plate provided in this kit has been pre-coated with coupled antigen. During the detection, TER in the samples or standard competes with coupled antigen on the solid phase supporter for sites of anti-TER 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 TER. The concentration of TER in the samples can be calculated by comparing the OD of the samples to the standard curve.

Technical indicator

Reaction mode (Incubation time and temperature)**:** 25℃, 30 min,15 min

Detection limit: Serum, Urine --- 0.5 ppb; Muscle, Liver ---1 ppb

Cross-reactivity:

Terbutaline	100%
Salbutamol	121%
Penbutolol	<0.1%
Fenoterol	<0.1%
Zilpaterol	<0.1%
Ractopamine	<0.1%
Dopamine Hydrochloride	<0.1%
Formoterol	<0.1%
Clenbuterol	<0.1%
Tulobuterol	<0.1%

Sample recovery rate: 90%±30%.

Kits components

Item	Specifications
ELISA Microtiter Plate	96 wells
Standard Liquid	1 mL each (ppb=ng/mL=ng/g) (0 ppb, 0.1 ppb, 0.3 ppb, 0.9 ppb, 2.7 ppb, 8.1 ppb)
Sample Diluent	30 mL
11×Concentrated HRP Conjugate	1 mL
HRP Conjugate Diluent	10 mL
Substrate Reagent A	6 mL
Substrate Reagent B	6 mL
Stop Solution	6 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, Vortex mixer, Water Bath, Centrifuge, Balance (sensibility 0.01 g).

Micropipette: Single channel (20-200 μL, 100-1000 μL), Multichannel (30-300 μL).

Reagents: Trichloroacetic Acid ($C_2HCl_3O_2$), NaOH

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. FOR RESEARCH USE ONLY. ELISA Microtiter plate should be covered by plate sealer. Avoid th e kit to strong light.
5. **Each reagent is optimized for use in the E-FS-E091. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other E-FS-E091 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. **For mentioned sample fast and efficient extraction methods are included in the kit descriptio n. Please consult technical support for the applicability if other sample need to be tested.**
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℃.

Expiry date: expiration date is on the packing box.

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**

   Please prepare solution according to the number of samples. Don't use up all components in the kit at once!

   Solution 1: Tissue Diluent *(for livestock, liver sample)*

   Dissolve 1 g of **Trichloroacetic Acid** with 100 mL of deionized water, mix fully.

   Solution 2: 0.5 M NaOH Solution *(for livestock, liver sample)*

   Dissolve 2 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).

   Solution 4: HRP Conjugate

   Dilute **11×Concentrated HRP Conjugate** with **HRP Conjugate Diluent**. (11× Concentrated HRP Conjugate (V): HRP Conjugate Dilution (V) = 1:10).

   Note: Please use immediately, it cannot be stored.

3. Sample pretreatment procedure

3.1 Pretreatment of serum (cattle, sheep) sample:

(1) Take 200 μL of serum into 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 vortex for 2 min;

(3) Incubate at 80℃ for 5 min in water bath;

(4) Restore samples to room temperature, and take 40 μL for detection.

Note: Sample dilution factor: 2, detection limit: 0.5 ppb

3.2 Pretreatment of muscle (livestock), liver sample:

(1) Homogenize the representative sample with a homogenizer and mix fully.

(2) Weigh 2±0.05 g of homogenate sample into a 50 mL centrifuge tube;

(3) Add 3 mL of **Wash Buffer** (Solution 3), then add 3 mL of **Tissue Diluent** (Solution 1) and fully vortex for 1 min;

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

(5) Take 1 mL of mediate layer clear liquid into a new centrifuge tube;

Note: Avoid taking the upper and lower solid, otherwise it will affect the test results.

(6) Add 40 μL of **0.5 M NaOH Solution** (Solution 2), and fully vortex for 10s to mix thoroughly;

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

(8) Take 40 μL for detection.

Note: Sample dilution factor: 4, detection limit: 1 ppb

3.3 Pretreatment of muscle (swine) sample:

(1) 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.

(2) Take 40 μL for detection.

Note: Sample dilution factor: 1, 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 40 μL of **Standard or Sample** per well, add 80 μL of **HRP Conjugate**（Solution 4) into each well. Gently oscillate for 10s to mix thoroughly and cover the plate with plate sealer. Incubate at 25℃ for 30 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. **Color Development:** add 50 μL of **Substrate Reagent A** to each well, and then add 50 μL of **Substrate Reagent B**. Gently oscillate for 10s to mix thoroughly. Incubate at 25℃ for 15 min in shading light (The reaction time can be extended according to the actual color change).
5. **Stop Reaction:** add 50 μL of **Stop Solution** to each well, oscillate gently for 10s to mix thoroughly.
6. **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/A_0 ×100%**

   A: Average absorbance of standard or sample

   A_0: 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.