SAL (Salbutamol) ELISA Kit

Catalog No: E-FS-E017

96T/96T*3

Version Number:	V1.2
Replace version:	V1.1
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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.

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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 Salbutamol (SAL) in samples, such as muscle, feed, urine, etc. This kit is composed of ELISA Microtiter plate, HRP conjugate, antibody working solution, standard and other supplementary reagents. The microtiter plate in this kit has been pre-coated with coupled antigen. During the reaction, SAL in the samples or standard competes with coupled antigen on the solid phase supporter for sites of anti-SAL antibody. Then Horseradish Peroxidase (HRP) conjugate is added to each microtiter plate well, and substrate reagent is added for color development. There is a negative correlation between the OD value of samples and the concentration of SAL. The concentration of SAL 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±2℃; 30 min, 15-20 min

Detection limit: Porcine urine, bovine urine, ovine urine, tissue---approx. 0.5 ppb; swine feed---approx. 20 ppb; bovine feed---approx. 10 ppb.

Cross-reactivity: Salbutamol---100%; Cimaterol, Terbutaline, Brombuterol, Clenbuterol, Ractopamine, Zilpaterol, Propranolol, Labetalol, Oxprenolol, Epinephrine, Atenolol, Vedoranolol, Clenpamine---< 0.1%

Sample recovery rate: 90% ± 30%.

Kits components

Item	Specifications
ELISA Microtiter plate	96 wells
Standard Liquid	1.5 mL each (ppb=ng/mL=ng/g) (0 ppb, 0.05 ppb, 0.15 ppb, 0.45 ppb, 1.35 ppb, 4.05 ppb)
HRP Conjugate Diluent	8 mL
11×Concentrated HRP Conjugate	1 mL
20×Concentrated Wash Buffer	25 mL
Sample Diluent A	4 mL
Sample Diluent B	4 mL
Sample Stabilizer	4 mL
Substrate Reagent A	7 mL
Substrate Reagent B	7 mL
Stop Solution	7 mL
Plate Sealer	1 piece
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

Instruments: Microplate reader, Printer, Homogenizer, Nitrogen evaporators, Water bath, Vortex mixer, Centrifuge, Graduated pipette, Balance (sensibility 0.01 g).

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

Reagents: NaOH, Conc. HCl, Trichloroacetic acid (TCA), $Na_2HPO_4 \cdot 12H_2O$, $NaH_2PO_4 \cdot 2H_2O$.

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 the kit to strong light.
5. **Each reagent is optimized for use in the E-FS-E017. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other E-FS-E017 with different lot numbers.**
6. Substrate Reagent should be abandoned if it turns blue color. When OD value of standard (concentration: 0) < 0.5 unit (A450nm < 0.5), it indicates the reagent 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 description. 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℃. After opening, the kit is stable for up to 1 month.

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**

   Solution 1: **Tissue Diluent**

   Dissolve 1 g of Trichloroacetic acid (TCA) with deionized water to 100 mL, mix fully.

   Solution 2: **Feed Diluent**

   Dissolve 4.37 g of $Na_2HPO_4 \cdot 12H_2O$ and 1.22 g of $NaH_2PO_4 \cdot 2H_2O$ with deionized water to 100 mL, mix fully.

   Solution 3: **1 M NaOH Solution**

   Dissolve 4 g of NaOH with deionized water to 100 mL, mix fully.

   Solution 4: **0.5 M NaOH Solution**

   Dissolve 2 g of NaOH with deionized water to 100 mL, mix fully.

   Solution 5: **0.1 M HCl Solution**

   Dilute 860 µL of Conc. HCl to 100 mL with deionized water, mix fully.

   Solution 6: **Wash Buffer**

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

   Solution 7: **HRP Conjugate**

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

3. **Sample pretreatment procedure**

3.1 Pretreatment of porcine urine, ovine urine sample:

(1) Take 1 mL of clarified fresh sample to detect (the turbid urine sample should be filtered or centrifuge at 4000 rpm for 5 min to get clear urine sample). Samples can be stored at 2-8℃ for one week.If samples are not tested within one week, store them at below -20℃ and avoid repeated freeze-thaw cycles.

(2) Add 20 µL of **Sample Diluent A** into the sample well before testing.

(3) Take 20 µL of urine for analysis.

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

3.2 Pretreatment of bovine urine sample:

(1) Take 1 mL of clarified fresh sample to detect (the turbid urine sample should be filtered or centrifuge at 4000 rpm for 5 min to get clear urine sample). Samples can be stored at 2-8℃ for one week.If samples are not tested within one week, store them at below -20℃ and avoid repeated freeze-thaw cycles.

(2) Add 20 µL of **Sample Diluent B** into the sample well before testing.

(3) Take 20 µL of urine for analysis.

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

3.3 Pretreatment of tissues (pork, pork liver, mutton, lamb liver, beef, pork brain) sample:

(1) Remove fat from sample, homogenize the sample with homogenizer.

(2) Weigh 2±0.05 g of homogenized fresh tissues sample into a 50 mL centrifuge tube, add 3 mL of **Wash Buffer** (Solution 6), then add 3 mL of **Tissue Diluent** (Solution 1), vortex thoroughly for 1 min, centrifuge at 4000 rpm at room temperature for 5 min.

(3) Take 1 mL of the middle-layer clear liquid into a new centrifuge tube *(Note: Avoid taking solids from the upper and lower layers; otherwise, the test results will be affected !)*, add 40 µL of **0.5 M NaOH Solution** (Solution 4), vortex thoroughly for 10 s, centrifuge at 4000 rpm at room temperature for 5 min.

(4) Take 40 µL of upper liquid for analysis.

Note: Sample dilution factor: 4, detection limit: 0.5 ppb

3.3 Pretreatment of swine feed, bovine feed sample:

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

(2) Weigh 1±0.05 g of homogenized sample into a 50 mL centrifuge tube, add 9 mL of **0.1 M HCl Solution** (Solution 5), vortex for 1 min, Centrifuge at 4000 rpm at room temperature for 5 min.

(3) Take 1 mL of the upper clear liquid into a new centrifuge tube, add 50 µL of **1 M NaOH Solution** (Solution 3), vortex thoroughly for 10 s, centrifuge at 4000 rpm at room temperature for 5 min.

(4) Take 100 µL of liquid to another centrifuge tube, add 400 µL of **Feed Diluent** (Solution 2), mix fully, vortex thoroughly for 30 s.

(5) Take 40 µL for analysis.

Note: Sample dilution factor: 50, detection limit: swine feed--20 ppb;bovine feed--10 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** (Sample : (1) Detection of porcine urine and ovine urine: First add 20 μL of Sample Diluent A to the sample well, then add 20 μL of urine sample; (2) Detection of bovine urine: First add 20 μL of Sample Diluent B into the sample well, then add 20 μL of urine sample; (3) Detection of tissues, swine feed and bovine feed: Add 40 μL of sample into the sample well) per well.
3. **HRP Conjugate:** then add 60 μL of **HRP Conjugate** (Solution 7) to each well, cover the plate with plate sealer, oscillate for 10 s gently to mix thoroughly, incubate at 25±2℃ for 30 min in shading light.
4. **Wash:** uncover the sealer carefully, remove the liquid in each well. Immediately add 260 μL of **Wash Buffer** (Solution 6) to each well and wash. Repeat wash procedure for 4 times, 15-30 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).
5. **Color Development:** add 100 μL of Substrate mixed solution to each well (**Substrate Reagent A** and **Substrate Reagent B** are fully mixed at ratio 1:1 by volume, the mixture should be used within 5 min, avoid using metal containers or stirring the reagents), Gently oscillate for 10 s to mix. thoroughly. Incubate at 25±2℃ for 15-20 min in shading ligh.
6. **Stop reaction:** add 50 μL of **Stop Solution** to each well, gently oscillate and mix fully.
7. **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 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 on a large number of samples.

Salbutamol (E-FS-E017) **Standard Curve**