



## Technical Manual

### SAL (Salbutamol) ELISA Kit

- Catalogue Code: FSES0014
- Competitive ELISA Kit
- Research Use Only

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## 1. Key features and Sample Types

### Sensitivity:

0.1 ppb (ng/mL)

### Assay Procedure:

25°C, 30 min~15 min

### Detection Limit:

Muscle (method 2), Urine - 0.1 ppb; Muscle (method 1) - 0.4 ppb; Feed - 1 ppb

### Cross Reactivity:

Salbutamol - 100%, Dobutamine Hydrochloride - 7.2%, Cimiterol - 0.1%

Clenbuterol - 3.6%, Ractopamine - 0.1%, Epinephrine - 0.1%, Isoprenaline < 1%

### Sample Recovery rate:

Urine - 90%±10%, Muscle - 80%±10%, Feed - 80%±15%

### Storage:

2-8°C for 6 months.

### Expiry:

See Kit Label

## 2. Storage

Store the kit at 2~8°C. 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

## 3. Test Principle

This kit uses a Competitive-ELISA method. 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.

## 4. Kit Contents

Each kit contains reagents for 96 assays including:

No.	Component	96-WellKit
1	ELISA Microtiter plate	96 wells
2	Standards	1 mL each (0ppb, 0.1ppb, 0.3ppb, 0.9ppb, 2.7ppb, 8.1ppb)
3	HRP Conjugate	5.5 mL
4	Antibody Working Solution	5.5 mL
5	Substrate Reagent A	6 mL
6	Substrate Reagent B	6 mL
7	Stop Solution	6 mL
8	20×Concentrated Wash Buffer	40 mL
9	10×Reconstitution Buffer	50 mL
10	Plate Sealer	3 pieces
11	Sealed Bag	1 piece
12	Manual	1 copy

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

### Additional materials required:

#### Other materials required but not supplied

- **Instruments:** Microplate reader, Printer, Homogenizer, Nitrogen evaporators, Water bath, Vortex mixer, Centrifuge, Graduated pipette, Balance (sensitivity 0.01 g).
- **Micropipette:** Single channel (20-200 µL, 100-1000 µL), Multichannel (30-300 µL).
- **Reagents:** NaOH, Ethyl acetate, HCl, Acetonitrile, Isopropanol (C<sub>3</sub>H<sub>8</sub>O), N-hexane, Na<sub>2</sub>SO<sub>4</sub>, Methanol.

## 5. Experimental Preparation

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 pre-treatment 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 M NaOH Solution (*for muscle sample*)

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

Solution 2: Reconstitution Buffer (*for muscle, feed sample*)

Dilute the **10×Reconstitution Buffer** with deionized water.

(2×Reconstitution Buffer (V): Deionized water (V)=1:9). The Reconstitution buffer can be store at 4°C for a month.

Solution 3: Wash Buffer

Dilute **20×Concentrated Wash Buffer** with deionized water.

(20×Concentrated Wash Buffer (V): Deionized water (V) = 1:19).

### 3. Sample pre-treatment procedure

*Targets may be distributed unevenly, resulting in no detection. To avoid this, ensure to take sufficient samples when sampling.*

#### 3.1 Pre-treatment of urine (swine)\* sample:

\*Data validated in swine urine but pre-treatment can be applied for urine samples of multiple species.

1. Take 50 µL of clear urine sample to detect (the turbid urine sample should be filtered or centrifuge at 4000 r/min for 5 min to get clear urine sample). Samples can be stored at 2~8°C for one week. If samples are not tested within the week, then they must be stored below -20°C. Avoid freeze-thaw cycles.

**Note: Sample dilution factor: 1, detection limit: 0.1 ppb**

#### 3.2 Pre-treatment of muscle (method 1) sample:

1. Weigh  $2 \pm 0.05$  g of homogeneous muscle samples, add 6 mL of **Reconstitution Buffer** (Solution 2), vortex thoroughly for 2 min, centrifuge at 4000 r/min at room temperature for 10 min (incubate the sample at 85°C for 10 min before centrifugation if there is a high content of fat in muscle sample).
2. Take 50 µL of upper liquid to analyse.

**Note: Sample dilution factor: 4, detection limit: 0.4 ppb**

#### 3.3 Pre-treatment of muscle (method 2) sample:

1. Weigh  $2 \pm 0.05$ g of homogeneous muscle samples, add 1 mL of **Reconstitution Buffer** (Solution 2), vortex thoroughly, add 4 mL of **Acetonitrile**, vortex thoroughly, add 1 mL of **Isopropanol**, vortex thoroughly for 5 min, centrifuge at 4000 r/min at room temperature for 10 min.
2. Take 3 mL of upper liquid to another centrifuge tube, add 50 µL of **1 M NaOH**

**Solution** (Solution 1), add 7 mL of **Ethyl acetate**, vortex thoroughly for 5 min, centrifuge at 4000 r/min at room temperature for 10 min. Take all upper liquid to dry at 56°C with nitrogen evaporators or water bath.

3. Dissolve the dried residual with 1 mL of **Reconstitution Buffer** (Solution 2), add 1 mL of **N-hexane**, mix 30s; centrifuge at 4000 r/min at 15°C for 5 min.
4. Take 50 µL of lower liquid to analyse.

**Note: Sample dilution factor: 1, detection limit: 0.1 ppb**

### 3.4 Pre- treatment of feed sample:

1. Weigh  $1 \pm 0.05$  g of homogeneous feed sample, add 10 mL of **Methanol** and 5g of **Na<sub>2</sub>SO<sub>4</sub>**, vortex for 2 min, Centrifuge at 4000 r/min at room temperature for 10 min.
2. Take 1 mL of upper liquid after centrifugation to another centrifuge tube, dry at 56°C with nitrogen evaporators or water bath. Dissolve the dried residual with 1 mL of **Reconstitution Buffer** (Solution 2), add 1 mL of **N-hexane**, mix 30s. Centrifuge at 4000 r/min at room temperature for 5 min.
3. Take 50 µL of lower liquid to analyse.

**Note: Sample dilution factor: 10, detection limit: 1 ppb**

## 6. Assay Procedure

Bring all reagents and samples to room temperature (25°C) 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°C.

1. **Number:** number the sample and standard in order (multiple well), and keep a record of standard wells and sample wells. **Standard and Samples must be tested in duplicate.**
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 plate sealer, vortex for 5s gently to mix thoroughly, incubate at 25°C for 30 min away from direct sunlight.
3. **Wash:** uncover the sealer carefully, remove the liquid in each well. Immediately add 300 µL of **Wash Buffer** (Solution 3) to each well and wash. Repeat wash procedure for 5 times, 30s intervals/time. Invert the plate and pat it against absorbent paper (If bubbles exist in the wells, clean tips can be used to prick them).
4. **Colour Development:** add 50 µL of **Substrate Reagent A** to each well, and then add 50 µL of **Substrate Reagent B**. Gently vortex for 5s to mix thoroughly. Incubate at 25°C for 15 min away from direct sunlight.
5. **Stop reaction:** add 50 µL of **Stop Solution** to each well, gently vortex and mix fully.
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 10 min after stop reaction.

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## 7. Data Analysis

### 1. Absorbance (%) = $A/A_0 \times 100\%$

A: Average absorbance of standard or sample

A<sub>0</sub>: 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.

## 8. 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°C.
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 FSES0014. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other FSES0014 with different lot numbers.**
6. Substrate Reagent should be abandoned if it turns blue colour. When OD value of standard (concentration: 0) < 0.5 unit (A<sub>450nm</sub> < 0.5), it indicates the reagents are 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.

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### **Assay Genie 100% money-back guarantee!**

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### **Contact Details**



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