

Salbutamol (SALB) ELISA Kit

rev 08/18

(Catalog # K4209-100, 100 assays, Store at 4°C)

I. Introduction:

Salbutamol is a short-acting β 2-adrenoreceptor agonist used as antiasthmatic. It can improve the animal of lean meat and reduce fat content by adding micro-Salbutamol into feed, with higher toxicity than Ractopamine, therefore, Salbutamol is banned from using. The method of instrumental analytical is major to detect Salbutamol, but it needs expensive instruments, professional operators and complex pre-treatment; While the method of enzyme linked immunoassay has advantages with simple, rapid, high sensitivity, good specificity and low cost. This kit is a detection product developed based on competitive ELISA technology, with operation time as short as 50 min and a sensitivity of 0.1 ppb, and linear range from 0.1 ppb to 8.1 ppb.

II. Application:

This ELISA kit is used for *in vitro* quantitative determination of Salbutamol
 Detection Range: 0.1 ppb to 8.1 ppb
 Sensitivity: 0.1 ppb
 Detection limitation: 0.5 ppb for swine urine, 0.3 ppb for tissue, and 5 ppb for feed.

III. Sample Type:

Swine urine, tissue (port, liver), feed

IV. Kit Contents:

Components	K4209-100	Part No.
Micro ELISA Plate	8 X 12 Strips	K4209-100-1
Standard	1 ml X 6	K4209-100-2
HRP-conjugate	7 ml	K4209-100-3
Antibody	7 ml	K4209-100-4
TMB substrate	12 ml	K4209-100-5
Stop Solution	10 ml	K4209-100-6
Sample Diluent (5X)	30 ml	K4209-100-7
Wash Buffer (10X)	30 ml	K4209-100-8
Extract	10 ml	K4209-100-9
Extraction Reagent	5 ml	K4209-100-10
Plate sealers	4	K4209-100-11

V. User Supplied Reagents and Equipment:

- NaCl
- Microplate reader capable of measuring absorbance at 450 nm
- 25°C incubator
- Precision pipettes with disposable tips
- Distilled or deionized water
- Clean eppendorf tubes and graduated cylinders for preparing standards or sample dilutions
- Absorbent paper

VI. Storage and Handling:

The entire kit may be stored at 4°C for up to 12 months from the date of shipment. Opened kit may be stable for 1 month at 4°C.

VII. Reagent and Sample Preparation:

Note: Bring all reagents to room temperature (20-25°C) 30 minutes before use.

Before using the kit, spin tubes and bring down all components to the bottom of tubes.

1. **Sample Diluent 1:** Dilute Sample Diluent (5x) with deionized or distilled water at a volume ratio of 1:4, namely 1 volume of Sample Diluent (5x) plus 4 volume of deionized or distilled water
2. **Sample Diluent 2 (for feed):** Take 100 mL of Sample Diluent 1, then add 4g NaCl, shake well.
3. **Extraction Solution 1 (for liver):** Dilute Extract with deionized or distilled water at a volume ratio of 1:99, namely 1 volume of Extract plus 99 volume of deionized or distilled water.
4. **Extraction Solution 2 (for feed):** Dilute Extract with deionized or distilled water at a volume ratio of 1:9, namely 1 volume of Extract plus 9 volume of deionized or distilled water.
5. **Wash Buffer (1X):** If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 10 mL of **Wash Buffer (10X)** into 90 mL deionized or distilled water to prepare 100 mL of **Wash Buffer (1X)**. Keep it at 4°C for one month.
6. **Standards Concentration:**

Standards	S0	S1	S2	S3	S4	S5
Concentration (ppb)	0	0.1	0.3	0.9	2.7	8.1

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7. Sample Preparation:

Note: The prepared sample maybe stored for up to one day at 2-8°C.

A. Swine urine

1. Bring the sample to room temperature.
2. Take 50 µl of sample for further analysis. If not clear, centrifuge at 4000 rpm for 10 min; then take 50 µL of supernatant sample or further analysis. (Diluent factor: 1)

B. Tissue (pork, beef)

1. Weigh 1 g of homogenized sample.
2. Add 2 ml of **Sample Diluent 1**, vortex for 10 min.
3. Centrifuge at 4000 rpm for 10 min
5. Take 50 µl of sample for further analysis. (Dilution factor: 3)

C. Feed

1. Weigh 2 g of the homogenized feed sample.
2. Add 10 ml of **Extraction Solution 2**, vortex for 10 min.
3. Centrifuge at 4000 rpm for 10 min.
4. Transfer 20 µl of supernatant into 180 µl of **Sample Diluent 2**, shake well.
5. Take 50 µl of sample for further analysis. (Dilution factor: 50)

D. Tissue (liver)

1. Weigh 1 g of homogenized sample.
2. Add 2 mL of **Extraction Solution 1**, vortex for 10 min. Centrifuge at 4000 rpm for 10 min
3. Take 950 µL of supernatant sample, then add 50 uL of **Extraction Reagent**, shake well.
4. Take 50 µL of sample or further analysis. (Dilution factor: 3)

VIII. Assay Protocol:

Note: Bring all reagents and samples to room temperature 30 minutes prior to the assay.

It is recommended that all standards and samples be run at least in duplicate.

A standard curve must be run with each assay.

1. Prepare all reagents, samples and standards as instructed in section VII.
2. Add 50 µl of **Standard** or **Sample** per well. Then add 50 µl of **HRP-conjugate** to each well and 50 µl of **Antibody** to each well. Cover the microtiter plate with a new adhesive strip and mix well, then incubate for 30 min at 25°C.
3. Aspirate each well and wash, repeating the process 4 times. Wash by filling each well with 250 µl of **Wash Buffer** using a squirt bottle, multi-channel pipette, manifold dispenser, or autowasher, and let it stand for 30 seconds, complete removal of liquid at each step is essential to good performance.
4. Add 100 µl of **TMB Substrate** to each well, mix well. Incubate for 15 minutes at 25°C. Protect from light.
5. Add 50 µl of **Stop Solution** to each well, gently tap the plate to ensure thorough mixing.
6. Read result at 450 nm within 10 minutes.

IX. CALCULATION:

The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The concentration of the samples can be interpolated from the standard curve. If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

X. RELATED PRODUCTS:

- Fluoroquinolones ELISA Kit (Cat. No. K4205-100)
- Aflatoxin B1 (AFB1) ELISA Kit (Cat. No. K4208-100)
- Sulfonamides residue ELISA Kit (Cat. No. K4207-100)
- AK Bioluminescence Cytotoxicity Assay Kit (Cat. No. K312-500)
- Gentamicin ELISA kit (Cat. No. K4206-100)

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