# Competitive enzyme immunoassay Kit for Quantitative Analysis of Salbutamol

## 1. Background

Salbutamol (SAL) is an adrenal gland receptor excitomotor (also known as nerve agonist) , which is applied in clinical therapy of bronchial asthma, chronic bronchitis and emphysema. This drug is also illegally employed in farm animals for improving lean meat percentage, decreasing fat aggradation and prompt animal growth. Its residue inside the body can cause human muscular tremor, palpitation, nervosity, headache and dizziness, etc., which is quite harmful to consumers. Now it is prohibited in animal husbandry .

HPLC or GC-MS is the common approach to detect β-agonist, and it is limited for the high expense and long time cost in sample pre-treatment. Enzyme-linked immunoassay is rapid and accurate, which can be used for SAL residue detection in urine, tissue, liver and feed samples.

## 2. Test Principle

This kit is based on indirect-competitive ELISA technology. The microtiter wells are coated with coupling antigen. SAL residue in the sample competes with the antigen coated on the microtiter plate for the antibody. After the addition of enzyme labeled anti-antibody, TMB substrate is used to show the color. Absorbance of the sample is negatively related to the SAL reside in it, after comparing with the Standard Curve, multiplied by the dilution factor, SAL residue quantity in the sample can be calculated.

## 3. Applications

This kit can be used in quantitative and qualitative analysis of SAL residue in animal tissue (muscle and liver),urine and feed samples.

## 4. Cross-reactions

Salbutamol( SAL)……………………………...……100%

Clenbuterol…………………………………………..…1%

Ractopamine……………………………………….…...1%

## 5. Materials Required but not Provided

### 5.1 Equipments

------Microtiter plate spectrophotometer (450nm/630nm)

------Rotary Evaporator/Nitrogen drying instrument

------Homogenizer ( or stamocher )

------Shaker

------Gyroscope ( or vortex mixer )

------Centrifuge

------Analytical balance (inductance: 0.01g)

------Graduated pipette: 10ml

------Rubber pipette bulb

------Volumetric flask: 100ml, 500ml, 1L

------Glass test tube: 10ml

------Polystyrene Centrifuge tube: 2ml, 50ml

------Micropipettes: 20μl-200μl, 200μl-1000μl
250ul-multipipette

### 5.2 Reagents

------Methanol (AR)

------n-hexane(AR)

------Trichloroacetic acid (TDA, AR)

------Deionized water

## 6. Kit Components

- Microtiter plate with 96 wells coated with antigen
- Dicloxacillin standard solutions. (1ml×6 bottles)
  **0 ppb, 0.1ppb, 0.3ppb, 0.9ppb, 2.7ppb, 8.1 ppb**
- Spiking standard solution: 1ml, **100ppb**
- Enzyme conjugate (1ml)….….....…………....red cap
- Antibody solution (7ml)……………..……green cap
- Solution A (7ml) ……………………...…….white cap
- Solution B (7ml) ………….………………..…red cap
- Stop solution (7ml) ……………..………yellow cap
- 20×Concentrated wash solution (40ml)
  ………………………….…………transparent cap
- Extraction solution (50ml)…………………blue cap

## 7. Reagents Preparation

**Solution** 1: Wash solution

Dilute the concentrated wash solution with deionized water in the volume ratio of 1:19(or according to the requirement), which will be used to rinse the plate. The diluted wash solution can be conserved for a month at 4℃.

**Solution** 2: 3% trichloroacetic acid

Dissolve 15g trichloroacetic acid(solid) with deionized water to 500ml.

### 8. Sample Preparations

#### 8.1 Notice and precautions for the users before operation

(a) Please use one-off tips in the process of experiment, and change the tips when absorb different reagent.
(b) Make sure that all experimental tools are clean.
(c)Complete the assay within 3h after the tissue sample preparation.

#### 8.2 Urine

----Take 50μl transparent urine sample for assay(please centrifuge: 3000g, 15℃, 10min),keep the unused samples in freeze.

#### 8.3 Tissue, liver

----Homogenize the sample;
----Take 2.0±0.05g homogenate into a 50ml polystyrene centrifuge tube, add 2ml 3% trichloroacetic acid (**solution 2**), vortex for 5min, then centrifuge: 3000g, 25-25℃, 5min.
----Take 500μl supernate, add 500μl extraction solution(**kit provided**) , mix completely (pH should be between 7 to 9, adjust with acid or alkali if pH is out of that range).
---- Take 50μl of the prepared solution for assay.

#### 8.4 Feed

----Take 1.0±0.05g feed sample, add 10ml methanol, vortex for 5min, then centrifuge: 3000g, 20-25℃, 5min.
----Transfer 1ml organic supernate(equal to 0.1g sample) to 10ml glass tube, dry under 50-60℃ nitrogen water bath flow.
----Add 1ml n-hexane, vortex for 30s, then add 1ml 3% trichloroacetic acid(**solution 2**), vortex for 1min.
---- Centrifuge: 3000g, 20-25℃, 5min.
----Remove organic supernate, take 150μl sustrate, add 450μl extraction solution(**kit provided**), mix completely(pH should be between 7 to 9 )
---- Take 50μl of the prepared solution for assay.

### 9. Assay process

#### 9.1 Notice before assay:

9.1.1 Make sure all reagents and microwells are all at room temperature (20-25℃).

9.1.2 Return all the rest reagents to 2-8℃ immediately after used.

9.1.3 Washing the microwells correctly is an important step in the process of assay; it is the vital factor to the reproducibility of the ELISA analysis.

9.1.4 Avoid the light and cover the microwells during incubation.

#### 9.2 Assay Steps

9.2.1 Take all reagents out at room temperature (20-25℃) for more than 30min, homogenize before use.

9.2.2 Get the microwells needed out and return the rest into the zip-lock bag at 2-8℃ immediately.

9.2.3 all reagents should be rewarmed before use.

9.2.4 **Number:** Number every microwell position and all standards and samples should be run in duplicate. Record the standards and samples positions.

9.2.5 **Add standard solution/sample**: Add 50 μl of standard solution or prepared sample to corresponding wells.

9.2.6 **Mixture of the enzyme conjugate and antibody solution**: Mix the enzyme conjugate and antibody in the volume ration of 1:10 (e.g. 0.5ml enzyme conjugate + 5ml antibody) completely. The mixture can't be conserved, please use immediately.

9.2.6 **Add enzyme conjugate/antibody mixture**: Add 50μl of the mixture per well, mix gently by shaking the plate manually and incubate for 30min at 25℃ with cover.

9.2.7 **Wash**: Remove the cover gently and pour the liquid out of the wells and rinse the microwells with 250μl diluted wash solution(**solution 1**) at interval of 10s for 3-4 times. Absorb the residual water with absorbent paper.

9.2.8 **Coloration**: Add 50μl solution A and 50μl solution B to each well. Mix gently by shaking the plate manually and incubate for 15 min at 25℃ with cover(see 12.8).

9.2.9 **Measure**: Add 50μl the stop solution to each well. Mix gently by shaking the plate manually and measure the absorbance at 450nm against an air blank (It's suggested measure with the dual-wavelength of 450/630nm. Read the result

within 5min after addition of stop solution) (We can also measure by sight without stop solution in short of the ELISA Reader)

## 10. Results

### 10.1 Percentage absorbance

The mean values of the absorbance values obtained from the standards and the samples are divided by the absorbance value of the first standard (zero standard) and multiplied by 100%.

$$\text{Absorbance}\ (\%) = \frac{B}{B_0} * 100\%$$

B ——absorbance of standards or samples

$B_0$ ——absorbance of zero standard

### 10.2 Standard Curve

---To draw a standard curve: The absorbance value of standards as y-axis, semi-logarithmic of the concentration of the standards (ppb) as x-axis.

---The salbutamol concentration of each sample (ppb), which can be read from the calibration curve, is multiplied by the corresponding dilution factor of each sample followed, and the actual concentration of sample is obtained.

**Notice:**

*Special software has been developed for result calculation,whichcanbeprovidedonrequest.*

**Dilution factor of samples:**

urine: 1
Tissue, liver : 4
Feed: 40

## 11. Sensitivity, accuracy and precision

Test Sensitivity**:** **0.1ppb** Detection limit

Urine…………………………………………… 0.3ppb
Tissue, liver………….……………………………..…0.5ppb
Feed……………………….………………………..…5ppb

**Accuracy**

urine………………………………………….....……75±15 %
Tissue, liver……………………………...…………90±20%
Feed…………………………………...........……90±20%

**Precision:**

Variation coefficient of the ELISA kit is less than 10%.

## 12. Notice

12.1 The mean values of the absorbance values obtained for the standards and the samples will be reduced if the reagents and samples have not been regulated to room temperature (20-25℃).

12.2 Do not allow microwells to dry between steps to avoid unsuccessful repetitiveness and operate the next step immediately after tap the microwells holder.

12.3. Homogenize each reagent before use.

12.4. Keep your skin away from the stop solution for it is the 2M $H_2SO_4$ solution.

12.5 Don't use the kits out of date. Don't exchange the reagents of different batches, for it will drop the sensitivity.

12.6 Storage condition:

Keep the ELISA kits at 2-8℃,do not freeze. Seal rest microwell plates Avoid straight sunlight during all incubations. Covering the microtiter plates is recommended for the standard sample and colorless chromogenic reagent are sensitive to light.

12.7 Indications for the reagents going bad:

Substrate solution should be abandoned if it turns colors.

The reagents may be turn bad if the absorbance value (450/630nm) of the zero standard is less than 0.5 (A450nm<0.5).

12.8 The coloration reaction needs 30 min after adding Solution A and Solution B. And you can prolong the incubation time ranges to 35min or longer if the color is too light to be determined., never exceed 40min,on the contrary, shorten the incubation time properly.

12.9 The optimal reaction temperature is 37℃. Higher or lower temperature will lead to the changes of sensitivity and absorbance values.

12.10 This kit is valid for 6 months, the production date in printed on the package.

## 13. Storage condition and storage period

Temperature: 2-8℃.
Storage period: 12months.

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