

Ractopamine ELISA Kit

Cat.#: DEIA028 Size: 96T

1. Background

Ractopamine belongs to β -agonists family. β -agonist is a class of nutrient repartitioning agents, which is a phenylethanolamine ramification structurally and functionally similar to adrenalin and noradrenalin, it can promote animal growth, decrease body fat content and improve lean meat percentage. It has been prohibited to promote animal growth in EU. Ractopamine is a nonprescription drug, which is much cheaper than clenbuterol, thus it is being illegally used quite often in animal production.

This kit is a new product based on ELISA technology, which is fast, easy, accurate and sensitive compared with common instrumental analysis, so it can considerably minimize operation error and work intensity.

2. Test Principle

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

3. Applications

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

4. Cross-reactions

Ractopamine.....	100%
Dobutamine	1%
Clenbuterol.....	<0.1%
Dopamine hydrochloride.....	<0.1%
Salbutamol.....	<0.1%

5. Materials required but not provided

5.1 Equipment

- Microtiter plate spectrophotometer (450nm/630nm)
- Rotary Evaporator / Nitrogen drying instrument
- Homogenizer
- Shaker
- Vortex mixer
- Centrifuge
- Analytical balance (inductance: 0.01g)
- Graduated pipette: 10ml
- Rubber pipette bulb
- Glass test tube: 10ml
- Volumetric flask: 100ml. 500ml
- Polystyrene Centrifuge tube: 50ml
- Micropipettes: 20 μ l-200 μ l, 100 μ l-1000 μ l
250 μ l-multipipette

5.2 Reagents

- Deionized water
- n-hexane (AR)
- Methanol (AR)
- Concentrated hydrochloric acid (HCl, AR)
- Sodium hydroxide (NaOH, AR)

6. Kit Components

- | Microtiter plate with 96 wells coated with antigen
- | Standard solutions (5 bottles \times 1ml/bottle)
0ppb,0.1ppb,0.3ppb,0.9ppb,2.7ppb
- | Spiking standard solution: (1ml/bottle) **100ppb**
- | Concentrate enzyme conjugate 1ml.....red cap
- | Antibody solution 10ml.....green cap
- | Substrate Solution A 7ml.....white cap
- | Substrate Solution B 7ml.....red cap
- | Stop solution 7ml.....yellow cap
- | 10 \times concentrated wash solution 40ml.... white cap
- | 2 \times concentrated extraction solution 50ml
.....blue cap

7. Solutions

Solution 1: 0.2M HCl

Dissolve 8.3ml concentrated HCl with deionized water, dilute to 500ml

Solution 2: 2% NaCl

Dissolve 2.0g NaCl with deionized water, dilute to 100ml.

Solution 3: 2%NaCl-0.2M HCl-Methonal

Mix 30ml 2% NaCl, 30ml 0.2M HCl, and 30ml methonal, mix completely.

Solution 4: 0.5M NaOH

Dissolve 2.0g NaOH with deionized water and dilute to 100ml.

Solution 5: Extraction solution

Dilute the 2×concentrated extraction solution with deionized water in the volume ratio of **1:1**, which will be used for sample extraction. This solution can be conserved for a month at 4 °C.

Solution 6 Wash solution

Dilute the 10×concentrated wash solution with deionized water in the volume ratio of **1:9**, which will be used to rinse the plate. This solution can be conserved at 4 °C for 1 month.

8. Sample Preparations

8.1 Notice and precautions before operation

(a) Please use one-off tips in the process of experiment, and change the tips when absorbing different reagent.

(b) Make sure that all experimental instruments are clean.

(c) Prepared sample should be tested within 30min.

8.2 Urine

----Take 200µl of clear urine sample

(if the sample is not clear, please centrifuge for 10min, at 15 °C, at 3000g, till the sample is transparent. Keep unused samples in freeze.)

----Add 200µl of extraction solution (**solution 5**), vortex completely for 1min;

----Take 20µl of the prepared solution for assay.

Dilution factor 2

8.3 Tissue (muscle and liver):

----Homogenize the test sample;

----Take 2.0±0.05g of homogenate into a 50ml polystyrene centrifuge tube, add 4ml of 2% NaCl-0.2M HCl- Methonal (**solution 3**), shake fiercely for 1min with a shaker;

----Centrifuge: 5min / room temperature (20-25 °C) / 3000g;

For liver sample: take 0.5ml supernate (don't take the impurity), add 20µl of 0.5M NaOH (**solution 4**), add 0.5ml of extraction solution(**solution 5**), mix completely;

For muscle sample: take 0.5ml of supernate (don't take the impurity), add 35µl of 0.5M NaOH (**solution 4**), add 0.5ml of extraction solution(**solution 5**), mix completely;

----Take 20µl of the prepared solution for assay.

Notice: *The sample cannot be stored after centrifuge, which should be tested immediately (important).*

Dilution factor 6

8.3 Feed

----Take 1.0±0.05g of feed into a 50ml polystyrene tube ;

----Add 10ml of methanol, shake for 1min;

----Centrifuge: 5min / room temperature (20-25 °C) / 3000g;

----Take 1ml of upper layer of organic phase into a 10ml glass tube, dry with 50-60 °C water bath under nitrogen gas stream;

----Add 1ml of n-hexane, vortex for 1min, and then add 1ml of extraction solution (**solution 5**), and vortex for 30s to dissolve evenly;

----Centrifuge: 5min / room temperature (20-25 °C) / 3000g;

----Remove the supernatant n-hexane layer. Take 20µl of the substrate layer for assay.

Dilution factor 10

9. Assay process

9.1 Notice before assay

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

9.1.2 Return all the rest reagents to 2-8 °C 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 °C) for more than 30min, shake gently before use.

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

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

9.2.4 **Add standard solution/sample:** Add 20µl of standard solution or prepared sample to corresponding wells;

9.2.5 **Mix antibody and enzyme conjugate:** Mix the antibody solution with concentrated enzyme conjugate concentrate in the volume ratio of **40:1**(*e.g. 10ml of the antibody + 0.25 ml of the concentrated enzyme conjugate*), mix completely.

Please notice: this mixed solution cannot be stored, which should be used for assay immediately.

9.2.6 **Add antibody-enzyme conjugate mixed solution:** Add 80µl of antibody-enzyme conjugate mixed solution into each well. Mix gently by rocking the plate manually and incubate for 30min at 25 °C with cover.

9.2.7 **Wash:** Remove the cover gently and pour the liquid out of the wells and rinse the microwells with wash solution(**solution 6**) at interval of 10s for 4-5 times. Absorb the residual water with absorbent paper (the rest air bubble can be eliminated with unused tip).

9.2.8 **Coloration:** Add 50µl of solution A and 50µl of solution B to each well. Mix gently by rocking the plate manually and incubate for 10min at 25 °C with cover.

9.2.9 **Measure:** Add 50µl of stop solution to each well. Mix gently by rocking the plate manually and measure the absorbance at 450nm (*It's suggested measure with the dual-wavelength of 450/630nm. Read the result within 5min after addition of stop solution.*)

10. Results

10.1 Percentage absorbance

The mean values of the absorbance values obtained for the standards and the samples are divided by the absorbance value of the first standard (zero standard) and multiplied by 100%. The zero standard is thus made equal to 100% and the absorbance values are quoted in percentages.

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

B —absorbance standard (or sample)

B₀ —absorbance zero standard

10.2 Standard Curve

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

----The ractopamine 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.

Please notice:

Special software has been developed for all data analysis, which can be provided on request.

11. Sensitivity, accuracy and precision

Test Sensitivity: 0.1 ppb

Detection limit:

Urine.....0.2ppb
Tissue.....0.6ppb
Feed.....1ppb

Accuracy:

Urine.....95±15%
Tissue.....95±15%
Feed..... 80±15%

Precision:

CV 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 °C).

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

12.3 Shake each reagent gently before use.

12.4 Keep your skin away from the stop solution for it is the 0.5M H₂SO₄ solution.

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

12.6 Keep the ELISA kits at 2-8 °C, do not freeze. Seal rest microwell plates, Avoid straight sunlight during all incubations. Covering the microtiter plates is

recommended.

12.7 Substrate solution should be abandoned if it turns colors. The reagents may be deteriorated if the absorbance value (450/630nm) of the zero standard is less than 0.5 ($A_{450nm} < 0.5$).

12.8 The coloration reaction needs 10min after the addition of solution A and solution B. You can prolong the incubation time to 15min or more if the color is too light to be determined. Never exceed 20min. On the contrary, shorten the incubation time properly.

12.9 The optimal reaction temperature is 25 . Higher or lower temperature will lead to the changes of sensitivity and absorbance values.

13. Storage

Storage: 2-8 , cool and dark place.

Validity: 12 months.