Ractopamine（Rac）ELISA Test Kit

Storage conditions

- The kit shall be stored at [2-8 ℃]. Expiry date: 12 months
- The opened Microelisa Stripplate can be stored at [2-8 ℃] and avoid damp. Use for at least 2 months.

Introduction

The Ractopamine (Rac) ELISA Test Kit is a competitive enzyme immunoassay for the quantitative analysis of ractopamine in feed, tissue, and urine.

Principle of test

The method of Ractopamine (Rac) ELISA Test Kit is based on a competitive colorimetric ELISA assay.

The coupling antigen of Ractopamine has been coated in the plate wells. During the analysis, sample is added along with the primary antibody specific for the target antigen. If the target is present in the sample, it will compete for the antibody, thereby preventing the antibody from binding to the coated antigen attached to the well. Then add secondary antibody, tagged with a Horseradish Peroxidase enzyme, targets the primary antibody that is complexed to the drug coated on the plate wells.

The resulting color intensity, after addition of substrates, has an inverse relationship with the Rac residues concentration in the sample.

Technique Data

- Kit sensitivity: 0.05 ppb (ng/ml)
- reactive mode: 25℃, 30min-15min
- Detection Limits:

Sample	Detection Limits
Urine	0.05ppb
Tissue (method 1)	0.2ppb
Meat (method 2)	0.05ppb
Liver (method 2)	0.1ppb
Feed	0.5ppb

- Cross-reaction rate:

Name	Cross-reaction rate
Ractopamine	100%
Dobutamine	<1%
Salbutamol	<0.1%
Clenbuterol	<0.1%

- Recovery rate:

Sample	Recovery rate
Urine	95%±10%
Tissue, Feed	90%±15%

Composition of the Kit

Reagent	Quantity
Microelisa Stripplate	8well×12strips
Standard: 0ppb, 0.05ppb, 0.15ppb, 0.45ppb, 1.35ppb, 4.05ppb	1×1.0ml
High standard 100ppb (red cap)	1×1.0ml
Antibody working solution (blue cap)	1×9ml
Enzyme conjugate (red cap)	1×5.5ml
Substrate A solution (white cap)	1×6ml
Substrate B solution (black cap)	1×6ml
Stop Solution (yellow cap)	1×6ml
concentrated Wash Solution 20× (white cap)	1×40ml
concentrated Redissolving solution 10× (yellow cap)	1×50ml
Instructions	1
Adhesive Membrane	1
Sealed bag	1

Materials required but not supplied

- Equipments: microplate reader(450nm), printer, mixer or stomacher, nitrogen-drying device, oscillator, centrifuge, measuring pipets, and balance with a reciprocal sensibility of 0.01g
- Micropipettors: single-channel 20 to 200µl and 100 to1000µl, and multi-channel 300µl
- Reagents: acetonitrile, methanol, N-hexane, anhydrous sodium sulfate.

Sample pre-treatmen

◆ **Instructions**

Labware must be clean and the use of disposable pipette tips to avoid contamination of interference results

◆ **Solution preparation before sample pre-treatment:**

Liquor 1: redissolving solution:

10 times dilute the 10× concentrated redissolving solution with deionized water to be used for sample redissolving; it can be stored at 4 ℃ environment up to a month.

◆ **Sample pretreatment step:**

1. Urine:

- Centrifuge 0.5 ml of the urine sample at room temperature at 4000 r/min for 5 minutes.
- Use 50 μL of the supernatant for the assay. Stored the unused sample at -20℃

dilution times of the sample: 1 detection Limits: 0.05 ppb

2. Tissue (method 1):

(Remove fat from the meat or liver)

- Weight 2.0 ± 0.05g homogeneous tissue samples into the centrifuge tube.
- Add 6ml redissolving solution, oscillate for 2min fully, centrifuge at room temperature at 4000 r/min for 10 min (If the grease content is higher in tissue samples, oscillating fully, and centrifuge after bathing at 85 ℃ water for 10 min)
- Use 50 μL of the supernatant for the assay.

dilution times of the sample: 4 detection Limits: 0.2 ppb

3. Tissue (Liver and Meat) (method 2):

(Remove fat from the meat or liver)

- Weight 2.0 ± 0.05g homogeneous tissue samples into the centrifuge tube.
- Add 8ml acetonitrile solution, oscillate for 2min fully, centrifuge at room temperature at 4000 r/min for 10 min
- Take 5ml supernatant into a glass tube and blow dry at 50 -60℃with nitrogen or air.

Sample of Meat :

1) Add 1ml redissolving solution, oscillate for 30s fully.
2) Use 50μl of the supernatant for the assay.

dilution times of the sample: 1 detection Limits: 0.05 ppb

Sample of Liver :

1) Add 2ml N-hexane, stop oscillating until dissolving fully.
2) Add 1ml deionized water, oscillate for 30s fully, centrifuge at room temperature at 4000 r/min for 10 min
3) Take 50μL lower layer, 50μL redissolving solution, mixing them.
4) Use50μL for the assay.

dilution times of the sample: 2 detection Limits: 0.1 ppb

4. Feed:

- Weight 1.0 ± 0.05g crushed homogeneous feed samples, add 10ml methanol and 5g Anhydrous sodium sulfate, oscillate for 2min fully, centrifuge at room temperature at 4000 r/min for 10 min
- Take 1ml supernatant into a glass tube and blow dry at 50 -60℃with nitrogen or air.
- Add 1ml redissolving solution to dissolve the dry residues, then add 1ml N-hexane , oscillate for 30s fully, centrifuge at room temperature at 4000 r/min for 10 min
- Use 50μL of the lower aqueous layer for the assay.

dilution times of the sample: 10 detection Limits: 0.5 ppb

Enzyme-linked immune test steps

- Take out the microtiter plate and the reagents required from 4 ℃ cold storage environment, put it in a place with room temperature for over 30 min. The wash buffer would crystallize in refrigeration, in order to make the Wash Buffer dissolved thoroughly, it needs indoor temperature. Shake the reagent bottle before usage. Take out required quantity of microwell plates and frames. The remaining strips are stored in the foil bag and zip-locked closed. Store the remaining kit in the refrigerator (2-8°C).
- **Before experiment:** dilute 40 ml of the concentrated washing buffer (20×concentrated) with the distilled or deionized water to 800ml (or just to the required volume) for using.

Step 1: Number: determine the number of well (samples and standards) to be used and store unused wells in 4 ℃. Every sample and standard must be parallel well (double well), and record their location

Step 2: Addition reaction: Add 50μl standard or sample into marked well, then add 50μl HRP-conjugate solution into each well, next, add 50μl antibody working solution into each well

Step 3: Incubate: Cover with the adhesive Membrane, oscillate gently for 5s and incubate for 30 min at 25℃

Step 4: Washing: Uncover the adhesive Membrane, discard liquid, pipette 250μl washing buffer to every well, still for 30s then drain, repeat 5 times, Pat the plate dry on a blotting paper

Step 5: Color: Pipette 50ul Substrate A solution, then pipette 50ul Substrate B solution to each well, oscillate gently for 5s, avoid the light preservation for 15 min at 25℃ (Do not put any substrate back to the original container to avoid any potential contamination. Any substrate solution exhibiting coloration is indicative of deterioration and should be discarded.)

Step 6: Stop the reaction: Pipette Stop Solution 50μl to each well, oscillate gently, stop the reaction (the blue change to yellow).

Step 7: Calculate: Read absorbance at 450nm with microplate reader (Recommend reading the OD value at the dual-wavelength 450/630nm).finish this step within 10min.

Interpretation of result

◆ **Calculate the percentage of absorbance value**

Percentage of absorbance value (%) = $\frac{A}{A0} \times 100\%$

A—the average (double wells) OD value of the sample or the standard solution;

A0—the average OD value of the 0 ppb standard solution.

◆ **Draw the standard curve and calculate**

- Take absorbance percentage of standards as Y-axis, the corresponding log of standards concentration (ppb) as X-axis.
- Draw the standard semilog curves with X-axis and Y-axis.
- Take absorbance percentage of samples substitute into standard curve, then can get the corresponding concentration from standard curve; last, Multiplied by the corresponding dilution times is the actual concentration of Rac of samples
- It is more convenient for a large amount of samples to use professional analyzing software to calculate, this will be accurate and rapid. (Welcome to contact us for this software)

Attention

- ✧ Before test, the reagents and samples should be balanced to room temperature (25℃).if below 25℃, it will lead to all the standard OD value is low
- ✧ In washing process, the dry micro plate will lead to the non-linear standard curves and undesirable reproducibility, so continue to next step immediately after washing.
- ✧ The reproducibility is largely determined by consistency of washing step, so please mix uniformly and wash thoroughly.
- ✧ On Incubate step, cover micro plates with adhesive Membrane to avoid light.
- ✧ Do not mix reagents with those from other lots
- ✧ Substrate A/B solution is colorless, if not, please discard.
- ✧ If absorbance value of 0ppb is below 0.5, it means that the reagent may be metamorphic.
- ✧ Stop solution is corrosives, please avoid contact with skin.

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