Ractopamine ELISA Test Kit(tissue,feed)

1. Principle

The test kit is based on the competitive enzyme immunoassay for the detection of Ractopamine in the sample. The conjugated antigens is pre-coated on the micro-well stripes. The Ractopamine in the sample and the coupling antigens pre-coated on the micro-well stripes compete for anti-Ractopamine antibodies. After the addition of the enzyme conjugate, the TMB substrate is added for coloration. The optical density (OD) value of the sample has a negative correlation with the Ractopamine concentration in the sample. The value is compared to the standard curve and the Ractopamine concentration is subsequently obtained.

2. Technical specifications

Sensitivity : 0.1 ppb

Detection limit

Meat 0.1ppb
Liver..... 0.2ppb
feed ..... 15 ppb

Recovery rate

Tissue(meat/liver),feed..... 80±10%

Cross-reactions

Dobutamine 10%
Clenbuterol ..... <0.1%
Salbutamol ..... <0.1%

3. Components

1） Micro-well strips: 12 strips with 8 removable wells each
2） 6× standard solution (1 mL each): 0 ppb, 0.1 ppb, 0.3ppb, 0.9 ppb, 2.7 ppb, 8.1 ppb
3） Enzyme conjugate (7 mL) ..... red cap
4） Antibody working solution (7 mL) .....blue cap
5） Substrate A solution (7 mL) ..... white cap
6） Substrate B solution (7 mL)..... black cap
7） Stop solution (7 mL) ..... yellow cap
8） 20× concentrated washing buffer (40mL) ..... white cap
9） 5× concentrated redissolving solution (50mL) ..... transparent cap

4. Materials required but not provided

1) **Equipments:** microplate reader, printer, mixer or stomacher, nitrogen-drying device, oscillator, centrifuge, measuring pipets, balance (a reciprocal sensibility of 0.01 g)
2) **Micropipettors:** single-channel 20-200 µL and 100-1000 µL, and multi-channel 250 µL;
3) **Reagents:** ethanenitrile(CH_3CN), N-hexane,concentrated HCL, deionized water

Skype:missyan819 **Tel:** 86-755-21567288 **Fax:** 86-755-28938800
Email/MSN: info@lsybt.com,lvshiyuan711@gmail.com **Website**: http://www.lsybt.com
Address: Rm.507, No.2, longgang Overseas Venture Park,Shenzhen,China. 518172

5. Sample pre-treatment

Instructions (The following points must be dealt with before the pre-treatment)

1) Only the disposable tips can be used for the experiments and the tips must be changed when used for absorbing different reagents;
2) Before the experiment, each experimental utensil must be clean and should be re-cleaned if necessary, in order to avoid the contamination that interferes with the experimental results.

Solution preparation before sample pre-treatment:

1) The 5× concentrated redissolving solution is diluted 1:4 with deionized water (1 mL 5× concentrated redissolving solution + 4 mL deionized water) before use.
2) 0.1M HCL:0.86mLconcentrated HCL+ deionized water to reach volume=100mL
3) CH_3CN-0.1M HCI solution: $V_{acetonitrile}$: $V_{0.1M\ HCl}$ = 84 : 16

5.1 Samples preparation

a)meat:

1 Weigh 2±0.05 g Homogeneous sample, add 8 mL CH_3CN-0.1M HCI solution,shake vigorously for 5 min, centrifuge at 4000 r/min at 15 ℃for 10 min.

2 Take 5 mL supernatant (upper layer), blow to dry with nitrogen at 56 ℃.

3 Add 1 mL of the diluted redissolving solution, shake vigorously for 30s,

4.Take 50 µL for analysis.

Fold of dilution of sample 1

b)liver:

1 Weigh 2±0.05 g Homogeneous sample, add 8 mL CH_3CN-0.1M HCI solution,shake vigorously for 5 min, centrifuge at 4000 r/min at 15 ℃for 10 min.

2 Take 5 mL supernatant (upper layer), blow to dry with nitrogen at 56 ℃.

3 Add 2 mL N-hexane, shake properly,add 1 mL of the diluted redissolving solution, shake vigorously for 30s, centrifuge at 4000 r/min at 15 ℃for 5 min, remove the supernatant.

4 Take 50 µL extracts of **meat** sample(lower layer), add 50 µL of the diluted redissolving solution, mix evenly

5 Take 50 µL for analysis.

Fold of dilution of sample 2

c) feed

1.Weigh 2±0.05 g feed sample, put into 40 mL centrifugal tube.

2. Add 10 mL of CH_3CN-0.1 M HCI solution: ($V_{acetonitrile}$: $V_{0.1M\ HCl}$ = 84 : 16), shake vigorously for 5 min, centrifuge at 4000 r/min for 10 min.

3 Take 2 mL of the clear liquid (upper layer), blow to dry with nitrogen.

4 Dissolve dry resdues in 1 mL N-hexane ,then add 2 mL of the diluted redissolving solution , shake vigorously for 30s, centrifuge at 4000 r/min for 10 min.

5. Remove the upper layer, take aqueous phase (the lower), diluted 1:29 (1 mL sample + 29 mL of the diluted redissolving solution),shake for 30s

6 Take 50 μL for analysis.

Fold of dilution of sample : 150

6. ELISA procedures

6.1 Instructions

1. Bring all reagents and micro-well strips to the room temperature (20-25 ℃) before use.
2. Return all reagents to 2-8 ℃immediately after use.
3. The reproducibility of the ELISA analysis, to a large degree, depends on the consistency of plate washing. The correct operation of plate washing is the key points in the procedures of ELISA.
4. For the incubation at constant temperatures, all the samples and reagents must avoid light exposure, and each microplate should be sealed by the cover membrane.

6.2 Operation procedures

1 Bring test kit to the room temperature (20-25 ℃) for at least 30 min, note that each liquid reagent must be shaken to mix evenly before use; put the required micro-well strips into plate frames. Re-sealed the unused microplate, stored at 2-8 ℃, not frozen.

2 Solution preparation:dilute 40 mL of the concentrated washing buffer (20×concentrated) with the distilled or deionized water to 800 mL (or just to the required volume) for use.

3 Numbering: number the micro-wells according to samples and standard solution; each sample and standard solution should be performed in duplicate; record their positions.

4 Add 50 μL of the sample or the standard solution into separate duplicate wells; add 50 μL of enzyme conjugate and 50 μL of the antibody working solution into each well, seal the microplate with the cover membrane, and incubate at 25 ℃for 30 min.

5 Pour liquid out of microwell , flap to dry on absorbent paper; add 250 μL/well of washing buffer , wash for 15-30 seconds, repeat 4-5 times, then take out and flap to dry with absorbent paper. (if there are the bubbles after flapping, cut them with the clean tips)

6 Coloration: add 50 μL of the substrate A solution, 50 μL of the B solution into each well. Mix gently by shaking the plate manually, and incubate at 25 ℃for 20 min at dark for coloration.

6 Determination:add 50 μL of the stop solution into each well. Mix gently by shaking the plate manually. Set the wavelength of the microplate reader at 450 nm to determine the OD value of every well. (Recommend to read the OD value at the dual-wavelength 450/630 nm within 5 min).

7. Result judgment

There are two methods to judge the results; the first one is the rough judgment, while the second is the quantitative determination. Note that the OD value of the sample has a negative correlation with the Ractopamine in the sample.

7.1 Qualitative determination

The concentration range (ng/mL) can be obtained form comparing the average OD value of the sample with that of the standard solution. Assuming that the OD value of the sample Ⅰ is 0.311, and

that of the sample Ⅱ is 0.715, the OD value of standard solutions is: 1.642 for 0 ppb, 1.200 for 0.1 ppb, 0.780 for 0.3 ppb, 0.454 for 0.9 ppb, 0.236 for 2.7 ppb, 0.142 for 8.1 ppb, accordingly the concentration range of the sample Ⅰ is 0.9 to 2.7 ppb, and that of the sample Ⅱ is 0.3 ppb to 0.9 ppb.

7.2 Quantitative determination

The mean values of the absorbance values obtained for the average OD value (B) of the sample and the standard solution divided by the OD value (B_0) of the first standard solution (0 standard) and subsequently multiplied by 100%, that is

$$\text{Percentage of absorbance value} = \frac{B}{B_0} \times 100\%$$

B—the average OD value of the sample or the standard solution

B_0—the average OD value of the 0 ng/mL standard solution

Draw the standard curve with the absorption percentages of the standard solutions and the semilogarithm values of the Ractopamine standard solutions (ng/mL) as Y- and X-axis, respectively. Read the corresponding concentration of the sample from the standard curve by incorporating its absorption percentage into the standard curve. The resulting value is subsequently multiplied by the corresponding dilution fold, finally obtaining the actual concentration of Ractopamine in the sample.

Using the professional analyzing software of this kit will be more convenient for the accurate and rapid analysis of a large amount of samples. (Please contact us for this software)

8. Precautions

1 Bring all reagents and micro-well strips to the room temperature (20-25 ℃).

2 Return all reagents to 2-8 ℃ immediately after use.

3 The reproducibility of the ELISA analysis, to a large degree, depends on the consistency of plate washing. The correct operation of plate washing is the key point in the procedures of ELISA.

4 For the incubation at constant temperatures, all the samples and reagents must avoid light exposure, and each microplate should be sealed by the cover membrane.

5 The room temperature below 20 ℃ or the temperature of the reagents and the samples being not returned to the room temperature (20-25 ℃) will lead to a lower standard OD value.

6 Dryness of the microplate in the washing process will be accompanied by the situations including the non-linear standard curves and the undesirable reproducibility; So continue to next step immediately after washing.

7 Mix evenly, otherwise there will be the undesirable reproducibility.

8 The stop solution is the 2 M sulfuric acid solution, avoid contacting with skin.

9 Do not use the kit exceeding its expiry date. The use of diluted or adulterated reagents from the kits will lead to the changes in the sensitivity and the detecting OD values. Do not exchange the reagents from the kits of different lot numbers to use.

10 Put the unused microplate into an auto-sealing bag. The standard substance and the colourless color former is light sensitive, and thus they cannot be directly exposed to the light.

11 Discard the colouration solution with any color that indicates the degeneration of this solution. The detecting value of the 0 standard solution of less than 0.5 indicates its degeneration.

9. Storage and expiry date

Storage: store at 2-8 ℃, not frozen.

Expiry date: 12 months; date of production is on box.

Shenzhen Lvshiyuan Biotechnology Co.,Ltd makes no warranty of any kind, either expressed or implied, except that the materials from which its products are made of standard quality. If any materials are defective, Lvshiyuan Biotechnology will provide a replacement product.There is no warranty of merchantability of this product, or of the fitness of the product for any purpose. Lvshiyuan Biotechnology shall not be liable for any damages, including special or consequential damage, or expense arising directly ot indirectly from the use of the this product.

Green Earth depends on everyone' efforts

"Build of green Earth needs the cooperation of you and me"

Skype:missyan819 **Tel:** 86-755-21567288 **Fax:** 86-755-28938800
Email/MSN: info@lsybt.com,lvshiyuan711@gmail.com **Website**: http://www.lsybt.com
Address: Rm.507, No.2, longgang Overseas Venture Park,Shenzhen,China. 518172