VERSION 1.01

RACTOPAMINE ELISA TEST KIT

MANUAL

ELISAKITS.ONLINE

By Immunomart

Ractopamine ELISA Test Kit

Catalogue Number. IP100039

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.

Technical specifications

Sensitivity: 0.05 ppb
Incubator temperature: 25℃
Incubator time: 30min～15min
Detection limit: Urine 0.1ppb, Tissue 0.2 ppb
Cross-reactions: Ractopamine 100%, Dobutamine <1%, Salbutamol <0.1%, Clenbuterol <0.1%
Recovery rate: Urine, tissue 60~120%

Components

1	**Micro-well strips**	**12 strips with 8 removable wells each**	
2	6× standard solution (1mL each)	0ppb, 0.05ppb, 0.15ppb, 0.45ppb, 1.35ppb, 4.05pp	
3	Enzyme conjugate	7ml	red cap
4	Antibody working solution	7ml	blue cap
5	Substrate A	7ml	White cap
6	Substrate B	7ml	Black cap
7	Stop solution	7ml	Yellow cap
8	20× concentrated washing buffer	15ml	white cap
9	2x concentrated extracting solution	50ml X 2	transparent cap

Materials required but not provided

Equipments: microplate reader, printer, homogenizer, nitrogen-drying device, oscillator, centrifuge, measuring pipets, balance (a reciprocal sensibility of 0.01 g)
Micropipettors: single-channel 20-200 µL and 100-1000 µL, and multi-channel 30-300 µL;
Reagents: NaOH, HCl

Sample pre-treatment

Instructions

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
Solution I 0.2M HCl:
Take 17.2mL HCl, add deionized water to 1L.
Solution II 1M NaOH:
Take 4g NaOH, add deionized water to 100mL.
Solution Ⅲ Sample extracting solution:
Dilute 2× concentrated extracting solution with deionized water at 1:1.

Samples preparation

a) Urine

Take 50 µL clear urine, directly detect it (If urine is muddy, must filter or centrifuge at 4000 r/min for 10 min, then take clear urine). Store at frozen environment if don't use.
Fold of dilution of sample: 1

b) Tissue

1. Weigh 2±0.05 g homogenized tissue sample into 50ml centrifuge tube, add 3 mL 0.2M HCl, shake thoroughly for 3 min.
2. Then add 600ul 1M NaOH and 2.4ml Sample extracting solution, shake thoroughly for 3 min, centrifuge at above 4000 r/min at room temperature (20-25 ℃) for 10 min.
3. Take 50 µL clear up-layer liquid for analysis. (Note: if there is fat, remove fat or separate fat, take clear liquid for analysis)
Fold of dilution of sample: 4

ELISA procedures

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.

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.
2. Put the required micro-well strips into plate frames. Re-sealed the unused microplate, stored at 2-8 ℃, not frozen.
3. Washing buffer prepare: dilute 15ml 20× concentrated washing buffer with deionized water at 1:19 (1 part 20× concentrated washing buffer + 19 parts deionized water), or prepare as quantity needed.
4. Numbering: number the micro-wells according to samples and standard solution; each sample and standard solution should be performed in duplicate; record their positions.

5. 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, mix gently by shaking the plate manually, and incubate at 25 ℃ for 30 min.
6. Pour liquid out of microwell, flap to dry on absorbent paper; add 250 µL/well of washing buffer, wash for 15-30 seconds, then take out and flap to dry with absorbent paper, repeat 4-5 times. (if there are the bubbles after flapping, cut them with the clean tips)
7. 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 15 min at dark for coloration.
8. 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).

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.

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 0.3, and that of the sample Ⅱ is 1.0, the OD value of standard solutions is: 2.243 for 0ppb, 1.816 for 0.05ppb, 1.415 for 0.15ppb, 0.74 for 0.45ppb, 0.313 for 1.35ppb, 0.155 for 4.05ppb, accordingly the concentration range of the sample Ⅰ is 1.35 to 4.05ppb, and that of the sample Ⅱ is 0.15ppb to 0.45ppb.

Quantitative determination

The mean values of the absorbance values obtained for the percentage of the average OD value (B) of the sample and the standard solution divided by the OD value (B0) of the first standard solution (0 standard) and subsequently multiplied by 100%, that is,

Percentage of absorbance value = $(B / B_0) \times 100\%$

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)

Precautions

1. The room temperature below 25 ℃ 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.
2. 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.

3. Mix evenly, wash plate completely, the reproducibility of the ELISA analysis, to a large degree, depends on the consistency of plate washing.
4. The stop solution is the 2 M sulfuric acid solution, avoid contacting with skin.
5. 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.
6. Put the unused microplate into an auto-sealing bag to re-seal it. The standard substance and the colourless color former is light sensitive, and thus they cannot be directly exposed to the light.
7. Discard the colouration solution with any color that indicates the degeneration of this solution. The detecting value of the 0 standard solutin of less than 0.5 (A450 nm< 0.5) indicates its degeneration.
8. The optimum reaction temperature is 25 ℃, and too high or low temperatures will result in the changes in the detecting sensitivity and OD values.

Storage: store at 2-8 ℃, not frozen.
Expiry date: 12 months; date of production is on box.