IMMUNOMART

VERSION 1.01

β-agonists ELISA TEST KIT

MANUAL

ELISAKITS.ONLINE

By Immunomart

β-agonists ELISA Test Kit

Catalogue Number. IP100041

Principle

The β-agonists test kit is based on the competitive enzyme immunoassay for the detection of β-agonists in the sample. The coupling antigen is pre-coated on the micro-well stripes. The β-agonists in the sample and the coupling antigens pre-coated on the micro-well stripes compete for the anti-β-agonists 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 β-agonists in the sample. This value is compared to the standard curve and the β-agonists residues is subsequently obtained.

Technical specifications

Sensitivity: 0.1 ppb
Incubation Temperature: 25℃
Incubation Time: 30min～30min～15min
Detection limit: Porcine urine 0.3ppb, Pork 0.6ppb
Cross-reaction rate: Clenbuterol 100%, Cimaterol<4%, Brombuterol 60%, Mabuterol 108%, Bambuterol<5%, Clorprenaline<1%, Salbutamol 75%, Terbutaline 25%, Penbutolol 19%, Tulobuterol 23%, Fenoterol<0.1%, , Ractopamine<0.1%
Recovery rate: Porcine urine, pork 90%±30

Components

1	Micro-well strips	12 strips with 8 removable wells each	
2	6× standard solution (1mL each)	0ppb, 0.1ppb, 0.3ppb, 0.9ppb, 2.7ppb, 8.1ppb	
3	Enzyme conjugate	7ml	red cap
4	Antibody working solution	10ml	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	40ml	white cap
9	5× sample extracting solution	50ml	transparent cap

Materials required but not provided

Equipments: microplate reader, printer, homogenizer, vortex, oscillator, centrifuge, Incubator, measuring pipets, balance (a sensibility reciprocal of 0.01 g)
Micropipettors: single-channel 20-200μL, 100-1000μL, and multi-channel 30-300μL;
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
1) 0.2M HCI: dissolve 17.2mL HCI in deionized water to 1L.
2) 1M NaOH: dissolve 4g NaOH in deionized water to 100 mL.
3) Sample extracting solution: 1 part 5X sample extracting solution + 4 parts deionized water, mix evenly.

Samples preparation

a) Porcine urine

Take 20 µL clear urine, directly detect it (If urine are 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) ***Pork***

1. Weigh 2±0.05g homogenized tissue sample into a 50ml centrifuge tube, add 3ml 0.2M HCl, shake for 3 min.
2. Then add 600ul 1M NaOH solution and 2.4ml Sample extracting solution, shake for 3min, centrifuge at 4000 r/min at room temperature (20-25 ℃) for 10 min.
3. Take 20µL upper-layer liquid for analysis.(Note: if there is fat layer after centrifuge, remove fat layer or seperate fat layer, 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 °C) 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 point in ELISA the procedures;
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 reagent must be shaken 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. Dilute the 40ml 20X concentrated washing buffer at 1:19 with deionized water (1 part 20X concentrated washing buffer + 19 part 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 20μL of the sample or the standard solution into separate duplicate wells, then add enzyme conjugate, 50ul/well; then add antibody working solution, 80μL/well. Mix gently by shaking the plate manually, seal the microplate with the cover membrane, incubate at 25 ℃ for 30 min.
6. Pour liquid out of microwell, add 250μL/well of washing buffer, wash for 4-5 times, 15-30s each time, then take out and flap to dry with absorbent paper(if there are the bubbles after flapping, cut them with the clean tips).
7. Coloration: add 50μL of substrate A, then add 50μL substrate B into each well. Mix gently by shaking the plate manually, and incubate at 25 ℃ for 15 min in the dark for coloration.
8. Determination: add 50μL of the stop solution into each well. Mix gently by shaking the plate manually. Set the wavelength of the microplate reader at 450nm to determine the OD value of every well. (Recommend to read the OD value at the dual-wavelength 450/630nm 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 β-agonists concentration in the sample

Qualitative determination

The concentration range (ng/mL) obtained from 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 0.1ppb, 1.415 for 0.3ppb, 0.74 for 0.9ppb, 0.313 for 2.7ppb, 0.155 for 8.1ppb, accordingly the concentration range of the sample Ⅰ is 2.7 to 8.1ppb, and that of the sample Ⅱ is 0.3 to 0.9ppb.

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 standard solutions and the semilogarithm values of β-agonists 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 dilution fold, finally obtaining β-agonists concentration in the sample.

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, otherwise there will be the undesirable reproducibility.
4.The stop solution is the 2 M sulfuric acid solution, avoid contacting with the 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 colorless 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 solution (0 ppb) of less than 0.5 indicates its degeneration.
8. The optimum reaction temperature is 25 ℃, and too high or too 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.