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User's Manual

Clenbuterol residues ELISA Kit

REF **DEIANJ44**

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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

This ELISA kit is used for quantitative determination of Clenbuterol residues.

Principles of Testing

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

Reagents And Materials Provided

1) Micro-well strips: 12 strips with 8 removable wells each
2) 7× standard solution
3) Enzyme conjugate
4) Antibody working solution
5) Substrate A
6) Substrate B
7) Stop solution

Materials Required But Not Supplied

1) Equipments: microplate reader, printer, homogenizer, nitrogen-drying device, vortex, oscillator, centrifuge, measuring pipets, balance (a sensibility reciprocal of 0.01 g)
2) Micropipettors: single-channel 20-200 µL, 100-1000 µL, and multi-channel 30-300 µl;
3) Reagents: Acetonitrile (CH3CN), NaOH, ethyl acetate, methanol, n-Hexane, HCl (approx 36.5%), Na2SO4(100%)

Storage

Storage: store at 2-8°C, not frozen.

Specimen Collection And Preparation

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) 0.1 M HCl: dissolve 0.86 mL HCl (approx 36.5%) in deionized water to 100 mL.
2) 0.1 M NaOH(for tissue samples): dissolve 0.4 g NaOH in deionized water to 100 mL.
3) CH3CN-0.1 M HCl solution(for tissue samples): VCH3CN :VHCl =84:16

1. Urine

Take 20 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

2. Tissue (liver, pork, etc.)

Method one

1) Weigh 2±0.05 g homogeneous sample, put it into a 50ml centrifuge tube, add 6 ml deionized water, shake thoroughly for 2 min, centrifuge at above 4000 r/min at room temperature (20-25°C) for 10 min.(Tips: If the fat content is higher in the sample, can firstly put it in the water bath at 85°C for 10 min after shaking, then centrifuge)
2) Take 20 µL clear supernatant for analysis.

Fold of dilution of sample: 4

Method two

1) Weigh 2±0.05 g homogeneous sample, put it into a 50ml centrifuge tube, add 6 mL CH3CN-0.1 M HCl solution, shake for 2 min, centrifuge at above 4000 r/min at room temperature (20-25°C) for 10 min.
2) Take 3 mL of clear supernatant, add 2 mL 0.1 M NaOH and 6 mL ethyl acetate, shake for 1 min, centrifuge at above 4000 r/min at room temperature (20-25°C) for 10 min. Take all supernatant (almost is clear), blow to dry with nitrogen or air at 50-60°C.
3) Add 1 mL deionized water, mix for 30s, redissolve residues properly.
4) Take 20 µL for analysis.

Fold of dilution of sample: 1

3. Feed

1) Grind sample, weigh 1.0±0.05 g, put it into a 50ml centrifuge tube, add 10 mL methanol, then add 5 g Na2 SO4, shake thoroughly for 2 min, centrifuge at above 4000 r/min at 15°C for 10 min.
2) Take 1 ml supernatant (must be clear), blow to dry with nitrogen or air at 50-60°C. Add 1 ml deionized water to dissolve the dry residue, then add 1 mL N-hexane. Mix properly for 30 seconds, centrifuge at above 4000 r/min at room temperature 15°C for 5 min. Remove up-layer organic phase.
3) Take down-layer phase 20 µL for analysis.

Fold of dilution of sample: 10

Assay Procedure

Instructions

1. Bring all reagents and micro-well strips to the room temperature (20-25°C).
2. Return all reagents to 2-8°C 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.

Operation procedure

1. Bring test kit to the room temperature (20-25°C) for at least 30 min, note that each reagent must be shaken evenly before use; put the required micro-well strips into plate frames. Re-sealed the unused microplate, stored at 2-8°C, not frozen.
2. Numbering: number the micro-wells according to samples and standard solution; each sample and standard solution should be performed in duplicate; record their positions.
3. Add 20 µL of the sample or the standard solution into separate duplicate wells, then add enzyme conjugate, 50 µL/well; and antibody working solution, 80 µL/well. Mix gently by shaking the plate manually, seal the microplate with the cover membrane, incubate at 25°C for 30 min.
4. Pour liquid out of microwell, flap to dry on absorbent paper; add 250 µL/well of deionized water, wash for 4-5 times, 15-30 s 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).
5. Coloration: add 50 µL of substrate A solution and 50 µL B solution into each well. Mix gently by shaking the plate manually, and incubate at 25°C for 15 min in the 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 dualwavelength 450/630 nm within 5 min).