Version 30-08/25

User's Manual

Clenbuterol ELISA Kit

REF **DEIA016**

Σ **96T**

RUO

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.

Creative Diagnostics

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

This test kit is based on the competitive enzyme immunoassay for the detection of Clenbuterol in the sample.

General Description

Clenbuterol, a β-agonist drug,was commonly added to animal feed to increase the lean meat ratio. However, its consumption through animal products can cause various human health issues, leading to a global ban. This kit offers a convenient, rapid, and sensitive method for detecting clenbuterol residues in a large number of samples.

Principles of Testing

In the sample, clenbuterol competes with antigens on the microplate for specific antibodies. The enzyme catalyzes color development, and the color intensity inversely indicates clenbuterol levels: darker colors mean lower levels, and lighter colors indicate higher levels.

Reagents And Materials Provided

Note: ppb=μg/kg

1. Standard working solutions: 6 bottles, 1.5 mL each, with concentrations of 0 ppb, 0.1 ppb, 0.3 ppb, 0.9 ppb, 2.7 ppb, and 8.1 ppb.
2. Microplate: 1 plate (8 wells×12 strips)
3. Conjugate concentrate: 1 bottle (11×, 1 mL)
4. Conjugate diluent: 1 bottle (9 mL)
5. Concentrated wash solution: 1 bottle (20×, 25 mL)
6. Concentrated tissue diluent: 1 bottle (50×, 30 mL)
7. Substrate A and B solutions: 1 bottle each (7 mL)
8. Stop solution: 1 bottle (7 mL)
9. Instructions
10. Plate cover film
11. Sealed bags

Materials Required But Not Supplied

1. Microplate reader (detection wavelength 450 nm, reference wavelength 630 nm or 620 nm)
2. Balance (precision: 0.01 g)
3. Centrifuge (above 4000 g)
4. Vortex mixer

5. Micropipette
6. Timer
7. Feed extraction solution: Weigh 1.36 g of potassium dihydrogen phosphate, add 1000 mL of deionized water, and mix well.
8. 0.1 M HCl solution: Measure 8.6 mL of concentrated hydrochloric acid and dilute to 1000 mL with deionized water.
9. 0.5 M NaOH solution: Weigh 2 g of sodium hydroxide, dilute to 100 mL with deionized water, and mix well.
10. 10% Tween 20: Weigh 1 g of Tween 20, add 9 mL of deionized water, and mix well.
11. Cooked meat extraction solution: Measure 400 mL of 0.1 M HCl solution, add 6 mL of 10% Tween 20, and mix well.

Storage

Store the kit at 2-8°C, avoiding freezing, with a shelf life of 1 year. Unused microplate strips should be sealed and stored at 2-8°C°C.

Specimen Collection And Preparation

1. Swine Urine (Dilution Factor: 1)

   Take 80 µL of the test urine sample for detection.

   Note: If turbid, filter or centrifuge at 4000 g for 5 min.
2. Bovine and Ovine Urine (Dilution Factor: 2)

   Take 500 µL of the test urine sample and add 500 µL of wash working solution, vortex for 1 min.

   Take 80 µL for detection.

   Note: If turbid, filter or centrifuge at 4000 g for 5 min.
3. Tissue (Pork, Beef, Mutton, Chicken, Duck, Rabbit) (Dilution Factor: 4)

   Weigh 2±0.05 g of homogenized sample into a 50 mL centrifuge tube.

   Add 6 mL of tissue diluent.

   Vortex for 2 min to fully disperse the tissue.

   Centrifuge at above 4000 g for 5 min.

   Immediately take 80 µL of the supernatant for detection.
4. Swine Liver (Dilution Factor: 4)

   Weigh 2±0.05 g of homogenized sample into a 50 mL centrifuge tube.

   Add 6 mL of 0.1 M HCl solution.

   Vortex for 2 min to fully disperse the tissue.

   Centrifuge at above 4000 g for 5 min.

   Take 1 mL of the supernatant into a new centrifuge tube and add about 100 µL of 0.5 M NaOH solution to adjust the pH to 6-8 (test with pH paper).

Vortex for 10 s.

Centrifuge at above 4000 g for 5 min.

Immediately take 80 μL of the supernatant for detection.

5. Feed (Dilution Factor: 20)
   Weigh 0.5±0.05 g of sample into a 50 mL centrifuge tube.
   Add 10 mL of feed extraction solution, vortex for 2 min.
   Centrifuge at above 4000 g for 10 min.
   Take 80 μL of the supernatant for detection.
6. Processed Meat (Dilution Factor: 3)
   Weigh 2±0.05 g of homogenized sample into a 50 mL centrifuge tube.
   Note: The sample must not be spoiled.
   Add 4 mL of cooked meat extraction solution and vortex vigorously for 2 min.
   Centrifuge at above 4000 g for 5 min.
   Take 80 μL of the middle layer clear liquid for detection.
   Note: Carefully aspirate the middle layer clear liquid to avoid upper-layer impurities.

Reagent Preparation

1. **Wash working solution**: Dilute the concentrated wash solution with deionized water at a 1:19 volume ratio.
2. **Tissue diluent**: Dilute the concentrated tissue diluent with deionized water at a 1:49 volume ratio.

Assay Procedure

1. Insert the required microplate strips into the microplate rack and record the positions of standards and samples. It is recommended to run duplicates. Seal unused strips in a bag and store at 2-8°C immediately.
2. Add 80 μL of each standard working solution/sample solution to the corresponding wells.
3. Prepare the conjugate working solution by diluting the conjugate concentrate with the conjugate diluent at a 1:10 volume ratio. Mix gently.
   Note: Prepare according to needs and use immediately.
4. Add 70 μL of the conjugate working solution to each well.
5. Cover the plate, vortex for 10 s, mix well, and incubate at room temperature (25±2°C°C) in the dark for 20 min.
6. Remove the cover.
7. Discard the liquid in the wells and add 260 μL of wash working solution to each well, washing 4 times for 15-30 s each time.
8. Discard the liquid and blot the plate on absorbent paper to dry.
9. Immediately add 100 μL of the mixed substrate A and B solution to each well.
   Note: Mix substrate A and B in a 1:1 volume ratio, use within 5 min, and avoid metal containers.

10. Cover the plate, vortex for 10 s, mix well, and incubate at room temperature (25±2°C) in the dark for 15-20 min.
11. Remove the cover, add 50 µL of stop solution to each well, and vortex for 10 s to mix.
12. Read the absorbance values at dual wavelengths (450 nm and 630 nm or 620 nm) within 5 min using a microplate reader.

Calculation

1. Calculate the absorbance percentage of each standard/sample as (average absorbance of standard/sample / average absorbance of zero standard) × 100%.
2. Plot a standard curve with absorbance percentage on the y-axis and the logarithm of standard concentrations on the x-axis.
3. Determine the residual concentration of the sample from the curve, multiply by the dilution factor for the actual residue level.
4. Note: It is recommended to use the dedicated analysis software for accurate results.

Typical Standard Curve

Standard (ppb)	0	0.1	0.3	0.9	2.7	8.1
Absorbance	2.292	1.667	1.134	0.588	0.287	0.110

Note: Input absorbance values, standard concentrations, and dilution factors into the software for automatic curve fitting and result calculation. Environmental factors and operational differences may affect absorbance values and curve shape.

Analytical Limitations: ewf4This kit is for screening purposes only. Positive results should be confirmed with instrumental methods like GC/MS or LC-MS/MS.

Performance Characteristics

Sensitivity: 0.1 ppb

Standard curve range: 0.1 ppb-8.1 ppb

B0 absorbance should be above 0.8.

Intra-plate error <5%, inter-plate error <10%.

Recovery rate with the provided method: 90%±30%.

Minimum detection limits:

Swine urine, pork, beef, mutton, chicken, duck, swine liver, processed meat, rabbit: approx. 0.5 ppb

Bovine and ovine urine: approx. 1 ppb

Feed: approx. 5 ppb

Note: ppb=µg/kg

Specificity

Clenbuterol: 100%

Salbutamol, Terbutaline, Cimaterol, Ractopamine, Mapreterol: <0.1%

Precautions

1. Read the instructions carefully before using the kit.
2. Do not use expired kits, and do not mix reagents from different batches.
3. Bring all kit components to room temperature (25±2°C) before use (takes about 2 h).
4. Restore samples to room temperature before processing and analyze immediately after processing to ensure accuracy.
5. Avoid using metal containers for reagent handling.
6. Shake reagents well before use, and avoid bubbles during mixing.
7. If substrate A or B turns blue before use or immediately after mixing, the reagent is spoiled.
8. Each step of adding solutions should not exceed 3 min.
9. Minimize airflow above the microplate during detection.
10. The stop solution contains strong acid. Rinse immediately if it contacts skin or clothing.
11. Do not reuse pipette tips.
12. Dispose of waste according to local regulations to prevent pollution.