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Clenbuterol ELISA Kit

Catalog No.: abx364826

Size: 96T

Storage: Store at 4°C.

Application: For quantitative detection of Clenbuterol in Urine, Muscle and Feed.

Sensitivity: 0.05 ppb (ng/ml)

Detection Limit: Muscle – 0.2 ppb; Urine – 0.1 ppb; Feed – 1 ppb; Liver – 0.5 ppb.

Sample Recovery Rate: Urine – 95 ± 10%; Muscle, Liver, Feed – 85 ± 15%.

Cross-reactivity: Clenbuterol – 100%, Terbutaline – < 1%, Mabuterol – < 1%, Brombuterol – < 1%, Salbutamol - <1%, Ractopamine - < 1%

Introduction: Clenbuterol is a small molecule used medically as a bronchodilator in asthma patients, and recreationally as a weight loss product and alternative to anabolic steroids. Clenbuterol induces these effects by binding to, and activating, β2-adrenergic receptors. It is also administered as a treatment for allergic respiratory disease in horses, as well as acting as a relaxant to aid the birthing process in cattle. Utilising Clenbuterol as a means to increase the muscle mass of livestock is widely prohibited to mitigate the risk of consuming contaminated meat.

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Principle of the Assay

This kit is based on indirect-competitive enzyme-linked immuno-sorbent assay technology. An antigen is pre-coated onto a 96-well plate. During the reaction, Clenbuterol in the samples, or standards, competes with the antigen fixed to 96-well plate. Avidin conjugated to Horseradish Peroxidase is then added to each well. After TMB substrate solution is added, only wells that contain Clenbuterol will produce a blue color product that changes into yellow after adding the stop solution. The intensity of the yellow color is inversely proportional to the Clenbuterol amount bound on the plate. The O.D. absorbance is measured spectrophotometrically at 450 nm in a microplate reader, and then the concentration of Clenbuterol can be calculated.

Kit components

1. Pre-coated 96-Well Microplate
2. Standards – 0 ppb, 0.05 ppb, 0.15 ppb, 0.45 ppb, 1.35 ppb, 4.05 ppb: 1 ml each
3. Detection Reagent A: 5.5 ml
4. Detection Reagent B: 5.5 ml
5. TMB Substrate A: 6 ml
6. TMB Substrate B: 6 ml
7. Stop Solution: 6 ml
8. Wash Buffer (20X): 40 ml
9. Reconstitution Buffer (10X): 50 ml
10. Plate Sealer: 3
11. Hermetic Bag: 1

Materials Required But Not Provided

1. 25-37°C incubator
2. Microplate reader (450 nm)
3. High-precision pipette and sterile pipette tips
4. Centrifuge and 50 ml centrifuge tubes
5. Absorbent filter papers
6. 100 ml and 1 L graduated cylinders
7. Nitrogen evaporator or water bath
8. Homogenizer
9. Vortex mixer
10. Balance (sensitivity 0.01 g)

Reagents Required But Not Provided

1. Sodium Hydroxide (NaOH)
2. Ethyl Acetate
3. Concentrated HCL.
4. Methanol
5. Deionized Water
6. Sodium Carbonate (Na_2CO_3)

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Protocol

A. Preparation of sample and reagents

1. Preparation of sample pretreatment solutions

Bring all reagents to room temperature prior to preparation.

- **0.1 M HCL Solution**
  Dilute 0.86 ml of Concentrated HCL in 100 ml of deionized water. Mix thoroughly.

- **2 M NaOH Solution**
  Dissolve 8 g of NaOH in 100 ml of deionized water. Mix thoroughly.

- **10% Na_2CO_3 Solution**
  Dissolve 50 g of Na_2CO_3 in 450 ml of deionized water. Mix thoroughly.

- **70% Methanol Solution**
  Dilute 100% Methanol in deionized water at a ratio of 7:3 to make the 70% Methanol Solution (i.e., Mix 70 ml of 100% Methanol with 30 ml of deionized water to prepare 100 ml of 70% Methanol Solution).

- **1X Reconstitution Buffer**
  Dilute the 10X Reconstitution Buffer 10-fold with deionized water to prepare the 1X Reconstitution Buffer solution (e.g., dilute 50 ml 10X Reconstitution Buffer in 450 ml deionized water to prepare 500 ml of 1X Reconstitution Buffer). The 1X Reconstitution Buffer solution can be stored at 4°C for up to one month.

- **1X Wash Buffer**
  Dilute the 20X Wash Buffer 20-fold with deionized water to prepare the 1X Wash Buffer solution (e.g., dilute 40 ml 20X Wash Buffer in 760 ml deionized water to prepare 800 ml of 1X Wash Buffer).

2. Sample pretreatment

Store samples to be assayed within 24 hours at 2-8°C. Alternatively, aliquot and store at -20°C or -80°C for long-term storage. Avoid repeated freeze-thaw cycles.

- **Muscle** Remove fat from the sample. Finely mince tissues and homogenize with a tissue homogenizer, mixing fully. Weigh 1 ± 0.05 g of homogenized tissue and add to a 50 ml centrifuge tube. Add 3 ml of Reconstitution Buffer. Vortex for 2 minutes, then centrifuge at 4000 RPM for 10 minutes. Incubate at 85 °C for 10 minutes prior to centrifugation if the sample contains a high fat content. Take 50 µl and assay immediately.
  Note: Sample dilution factor: 4, minimum detection dose: 0.2 ppb.

- **Liver:** Remove fat from the sample. Finely mince tissues and homogenize with a tissue homogenizer, mixing fully. Weigh 1 ± 0.05 g of homogenized tissue and add to a 50 ml centrifuge tube. Add 1 ml of 10% Na_2CO_3 solution. Vortex for 2 minutes, then add 4 ml of Ethyl Acetate. Vortex for 5 minutes, then centrifuge at 4000 RPM for 10 minutes at room temperature. Take 2 ml of the supernatant and add to a 50 ml centrifuge tube. Add 1 ml of 0.1 M HCL Solution. Vortex for 2 minutes, then centrifuge at 4000 RPM for 5 minutes at room temperature. Aspirate 0.1 ml of the lower liquid and add 0.4 ml of Reconstitution Buffer. Add 2 M NaOH to adjust the pH to 7. Take 50 µl for analysis.
  Note: Sample dilution factor: 10, minimum detection dose: 0.5 ppb.

- **Urine**: Aliquot 1 ml of fresh urine into a 50 ml centrifuge tube. If the urine is not clear, filter and centrifuge at 4000 RPM for 5 minutes. Add 1 ml of Reconstitution Buffer. Vortex for 1 minute. Aliquot 50 µl and assay immediately.
  Note: Sample dilution factor: 2, minimum detection dose: 0.1 ppb.

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- **Feed:** Weigh 1 ± 0.05 g of homogenized food sample and add to a 50 ml centrifuge tube. Add 4 ml of 70% Methanol Solution. Vortex for 2 minutes, then centrifuge at 4000 RPM for 10 minutes at room temperature. Aspirate 0.1 ml of supernatant and add 0.4 ml of 1X Reconstitution Buffer. Take 50 µl for analysis.
  Note: Sample dilution factor: 20, minimum detection dose: 1 ppb.

Note:

» Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.

» Samples should be clear and transparent. Samples must be diluted so that the expected concentration falls within the kit's range.

» Please bring sample slowly to room temperature. Sample hemolysis will influence the result. Hemolyzed specimen should not be used. Samples that contain NaN_3 cannot be detected as it interferes with HRP.

» Always use non-pyrogenic, endotoxin-free tubes for blood collection.

B. Assay Procedure

Bring all reagents to room temperature prior to use.

1. Set standard, test sample and control (zero) wells on the pre-coated plate and record their positions. It is recommended to measure each standard and sample in duplicate.
2. Add 50 µl of the standard solutions into the standard wells.
3. Add 50 µl of prepared sample into test sample wells.
4. Immediately add 50 µl of Detection Reagent B, then 50 µl of Detection Reagent A to each well. Add the solution at the bottom of each well without touching the side wall.
5. Cover the plate with the plate sealer. Tap the plate gently to ensure thorough mixing. Incubate at 25°C for 30 minutes in dark conditions.
6. Remove the cover and discard the liquid. Wash the plate 5 times with 1X Wash Buffer. *Fill each well completely with 1X Wash Buffer (300 µl) using a multi-channel pipette or automated washer (0.5-2 mins soaking period is recommended). Complete removal of liquid at each step is essential for good performance. After the final wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean absorbent paper towels.*
7. Add 50 µl of TMB Substrate A, followed by 50 µl of TMB Substrate B into each well. Tap the plate gently to ensure thorough mixing. Cover the plate and incubate at 25°C in dark conditions for 15-20 minutes (incubation time is for reference only, do not exceed 30 minutes). When an apparent gradient appears in the standard wells the reaction can be terminated.
8. Add 50 µl of Stop Solution into each well. Mix thoroughly.
9. Ensure that there are no fingerprints or water on the bottom of the plate, and that the fluid in the wells is free of bubbles. Measure the absorbance at 450 nm immediately (dual wavelength at 450 nm / 630 nm).

This assay is competitive, therefore there is an inverse correlation between Clenbuterol concentration in the sample and the absorbance measured. Create a graph with the log of the standard concentration (y-axis) and absorbance measured (x-axis). Apply a best fit trendline through the standard points. Use this graph calculate sample concentrations based on their OD values. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Note: If the samples measured were diluted, multiply the dilution factor by the concentrations from interpolation to obtain the concentration before dilution.

C. Precautions

1. Equilibrate all reagents to room temperature (18-25°C) prior to use and centrifuge the tubes briefly in case any contents are trapped in the lid.
2. The concentrated Wash Buffer may crystallize and separate. If this happens, please warm the tube, and mix gently to dissolve.

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3. Avoid foaming or bubbles when mixing or reconstituting components.
4. Do not leave the wells uncovered for extended periods between incubations. The addition of reagents for each step should not exceed 10 mins.
5. Ensure that the plate is properly sealed or covered during the incubation steps, and that the time and temperature are controlled.
6. Complete removal of all solutions and buffers during wash steps is necessary for accurate measurement readings.
7. To avoid cross-contamination, do not reuse pipette tips and tubes.
8. Do not use expired components, or components from a different kit.
9. The substrate reagents should be used under sterile conditions, and light exposure should be minimized. Unused substrate should be colorless, or a very light yellow in appearance. Do not discard any residual solution back into the vial.

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