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QUICKING CLENBUTEROL ELISA KIT (W81111)

Enzyme immunoassay for in vitro diagnostic use

1. INTENDED USE

Clenbuterol ELISA Kit is a direct method competitive enzyme-linked immunoassay for the quantitative detects the presence of Clenbuterol residue in samples of urine, serum, feed and tissues such as muscle, liver, kidney.

Incubation Time: 30min + 15min

Assay Time Requirement: For urine and serum, approximately 1 hour; For feed, less than 2 hours; for muscle, liver, kidney, less than 2 hours.

Detection Limit: Clenbuterol 0.1 ppb (0.1ng/ml).

Detection Range: Urine/serum 0.5-40.5ppb; Feed 5-405ppb; Muscle/liver/kidney 0.1-8.1ppb.

Cross-reactivity: Clenbuterol 100%

Clenbuterol	100%	Salbutamol	<1%
Salmeterol	<0.1%	Terbutaline	<0.1%
Ractopamine	<0.1%	Adrenaline	<0.1%

Recovery Rate: Urine $85 \pm 15\%$; feed $85 \pm 15\%$; muscle/liver/kidney $85 \pm 15\%$

Precision: Intra-assay CV<6% Inter-assay CV<10%

2. PRINCIPLE OF THE ASSAY

Clenbuterol ELISA kit is a direct method competitive enzyme-linked immunoassay. The assay is performed in ELISA microwells, which has been precoated with one anti-Clenbuterol antibodies. When standards or samples are added into the microwells, the free Clenbuterol antigens and the enzyme conjugate will compete to combine the antibody binding site on the microwells during incubation. After that, the unbound materials will be removed in a washing step. Then, two substrates are added into the microwells. The bound enzyme conjugate will convert the colorless substrate into blue. The color will change from blue to yellow after adding stop solution. Absorbance is then measured spectrophotometrically (450 nm) and color intensity results are

inversely proportional to the original beta-agonists amount in the sample. Sample concentrations are then calculated on the basis of a calibration curve derived from the standards

3. KIT COMPONENT

- 1X Microtiter plate (8 microwells X 12 removable strips) precoated with anti-Clenbuterol antibodies
- 6X Clenbuterol standard solutions (1mL each): 0 ng/ml, 0.1ng/ml, 0.3ng/ml, 0.9 ng/ml, 2.7ng/ml, 8.1ng/ml
- 1X Clenbuterol Standard solution, (1mL): 100ng/mL
- 1X Enzyme conjugate, (13mL)
- 1X Washing buffer, 10X concentrate, (50mL)
- 1X Substrate A: Carbamide Peroxide, (6mL)
- 1X Substrate B: TMB solution, (6mL)
- 1X Stopping solution, 2M Sulphuric acid, (6mL)
- Product Manual
- QC Report

4. MATERIALS REQUIRED BUT NOT PROVIDED

- ELISA Microtiter plate reader equipped with 450nm and 620nm filters
- 50 - 200 µl multichannel micropipette
- 50, 100 and 200 µl precision micropipette
- Microplate washer or squeeze bottle
- Nitrogen gas
- Mortar
- Beakers
- Centrifugal tube
- Ethyl acetate
- Hexane
- 0.1M HCl solution
- 0.1M NaOH solution
- Distilled water

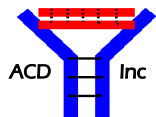
5. PREPARATION OF WORKING SOLUTIONS

Washing buffer: dilute 10X concentrate with distilled water (i.e. 1mL + 9mL). In case of precipitates, bring the buffer to room temperature and mix well into solution.

Standard solution, Enzyme conjugate, Extract liquid, substrate A, substrate B and stopping solution are ready-to-use

6. SAMPLE PREPARATION

- 6.1 Urine and Serum (dilution factor 5)
- Dilute 1/5 (100ul+ 400ul) to the sample with distilled water
 - Use the clean solution directly in the assay
- 6.2 Feed (dilution factor 50)
- Finely grind and pulverize the feed into a fine powder
 - Weigh 1g of the feed powder into a 50mL tube
 - Add 10 mL of 0.01M HCl solution and shake for 5min
 - Adjust the pH value to the range 6.5~8.0 with 0.1M HCl solution and 0.1M NaOH solution.
 - Do filtration or centrifuge to make the solution clean.
 - Collect 100uL of the supernatant into a tube.
 - Make dilution with 400uL of distilled water.
 - Use the solution in the assay.
- 6.3 Tissues (Muscle/Liver/Kidney) (dilution factor 1)
- Homogenize the sample sufficiently with a suitable mixer
 - Weigh 2g of homogenized sample into a 20 ml centrifugal tube
 - Add 3 ml of distilled water and 0.3mL 1M NaOH solution; mix well
 - Add 8mL of ethyl acetate; shake for 5 minutes
 - Do centrifugation at 10000rpm for 5 minutes.
 - Collect 4mL of the clean supernatant into a beaker
 - Dry the beaker by blowing nitrogen gas



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- Redissolve the residue in the beaker with 0.4mL of distilled water and transfer to a centrifugal tube
- Add 0.4 mL of hexane and shake for 1min
- Get rid of the supernatant. Use the clean under layer solution in the assay

7. ELISA TESTING PROTOCOL

7.1 Assay Preparation

- Bring all reagents to room temperature (1-2hours at 20-25°C) before use.
- Predispose a duplicate for each standard point and a duplicate for each sample

7.2 Testing procedures

- Place 20uL of each standard into the standard wells
- Place 20uL of each sample into the sample wells
- Place 100uL of enzyme conjugate solution into each well
- Mix gently by rocking the plate manually
- Incubate 30 minutes at room temperature (20-25°C)
- Pour the liquid out of the wells and tap the microwells holder upside down against absorbent laboratory paper to ensure complete liquid removal.
- Fill completely all the wells with washing buffer (approx 300uL/well) ;repeat the washing step 5 times. After the last washing step, tap the microwells holder upside down against absorbent paper to ensure complete liquid removal.
- Add 50uL of substrate A solution into each well
- Add 50mL of substrate B solution into each well
- Tap the microwells holder to make them mix thoroughly
- Incubate 15 minutes at room temperature (20-25°C)
- Add 50uL of stopping solution into each well. Mix gently by rocking the plate manually
- Read the absorbance at 450nm filter with a Microplate reader in 5 minutes

8. CALCULATION

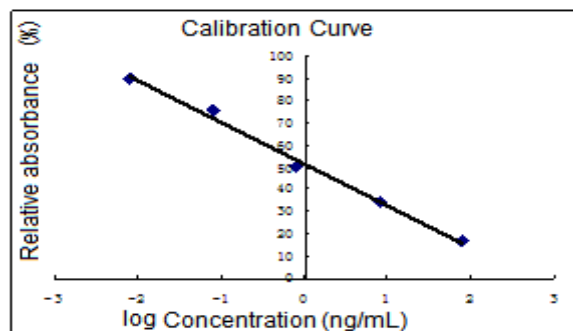
The unknown values for Clenbuterol concentration in samples are determined from a calibration curve.

- Calculate the mean absorbance value for zero standard and subtract it from the absorbance value of all other wells. (This step is commonly skipped in practical operation)
- Calculate the mean absorbance value for the Maximum Binding, the standards and the samples
- Calculate the relative absorbance

$$\text{Relative absorbance} = \frac{\text{Absorbance standard (or sample)}}{\text{Absorbance zero standard}} \times 100 = \frac{B}{B_0} (\%)$$

A special program with Excel functionality, Quicking ELISA Analysis Program in Excel, is available for giving the ELISA test results.

- Enter the Relative absorbance values calculated for each standard in a semi-logarithmic system of coordinates against the standard concentration; draw the calibration curve
- Take the relative absorbance value for each sample and interpolate the corresponding log concentration from the calibration curve. Calculate the actual concentration of the sample by multiplying the dilution factor to the extraction concentration.



9. STORAGE

- The kit should be stored at 2~8°C, NO FREEZING
- Unused test wells should be sealed and dryly stored

10. PRECAUTIONS

- Please carefully read the instruction before use
- Reagents should be brought to room temperature, 20-25°C prior to use
- Do not use reagents after expiration date; Do not misuse reagents from other kits with different Lot numbers; Do not substitute reagents from any other manufacturer into the test kit.
- Avoid contact of skin and mucous membranes with reagents and sample extraction; If exposure should occur, please immediately flush with water

11. LIMITATION

Clenbuterol ELISA kit is used as a screening system. If a more accurate result is required, other method such as HPLC or GC/MS is suggested to give a confirmation.