Biorex food diagnostics

BETA AGONIST ELISA

PRODUCT CODE: BXEFT05A

QUALITY MANAGEMENT SYSTEM
ISO 13485 CERTIFIED COMPANY

BXEFT05A
96 TESTS (12x8 STRIP WELLS)
STORE AT 2-8°C
INSTRUCTIONS FOR USE
FOR VETERINARY USE AND/OR USE IN THE ANALYSIS OF FOOD ONLY. Not for use in diagnostic procedures.

LOT Lot Number
REF Catalogue Number
Storage Temperature
Expiry Date (Year / Month)
Warning, Read Enclosed Documents
Instructions For Use
Manufactured By

BETA AGONIST ELISA

Introduction
Enzyme-Linked Immunosorbent Assay for the Determination of Beta Agonists in urine, muscle, feed and liver samples.

Importance of Beta Agonist determination
Beta agonists are growth hormones used in cattle and swine production. When animals consume feed, they partition the extra energy into fat cells. When cattle and swine are given beta agonists, they partition the extra energy into muscle instead of fat. Beta agonists are a bronchodilator medicine that opens the airways by relaxing the muscles around the airways that may tighten during an asthma attack or in chronic obstructive pulmonary disease. Beta-agonists can be administered by inhalers or orally. Beta-agonists promote heavier, leaner carcasses, providing less expensive meat and healthier choices. The side effects of beta agonists include anxiety, tremor, palpitations or fast heart rate, and low blood potassium.

Samples
Urines, muscle, liver and feed.

Sample Preparation
Urine – centrifuge, dilute and apply
Liver/muscle/feed – dilution using acidic buffer, centrifuged and neutralised prior to applying sample to test.

Assay Time
80mins

Sensitivity/Cut off/Limit of detection
Urine 0.185ppb
Muscle 0.092 ppb
Liver 0.3 ppb
Feed 3.7 ppb.

Specificity

Clenbuterol	100%
Cimbuterol	96%
Salbutamol	80%
Bromobuterol	77%
Mabuterol	55%
Carbuterol	55%
Clenpenterol	55%
Mapenterol	45%
Clenproperol	35%
Terbutaline	30%
Cimaterol	11%

Test Principle
The assay is performed in plastic microwells which have been coated with rabbit anti-β-agonists IgG. Clenbuterol standard or the sample solution, the enzyme conjugate and rabbit anti-β-agonists antibodies are added to the microwells. During the first incubation, free and enzyme-labelled β-agonists compete for the anti-β-agonists antibodies binding sites. Any unbound enzyme conjugate is then removed in a washing step. The bound enzyme activity is determined by adding a fixed amount of a chromogenic substrate. The enzyme converts the colourless chromogen into a blue product. The addition of the stop reagent leads to a colour change from blue to yellow. The absorbance is measured by a microplate reader at 450nm. The colour develoment is inversely proportional to the β−agonist concentration in the sample.

Reagents Provided

Code / Size	Component / Description
FT05-001 **FT05-002** **FT05-003** **FT05-004** **FT05-005** **FT05-006** (6 vials with 1.5mL each)	STANDARD A – 0.0 ng/ml STANDARD B – 0.037 ng/ml STANDARD C – 0.075ng/ml STANDARD D – 0.15ng/ml STANDARD E – 0.6 ng/ml STANDARD F –2.5 ng/ml Ready-to-use
FT05-008 (1x96 wells) 8×12/12×8 well per plate	**MICROTITER PLATE** consisting of 12 strips with 8 breakable wells each, coated with rabbit anti- β-agonists antibodies
WS-009 (1x20ml) ***DILUTE BEFORE USE***	**WASH BUFFER:** Wash Buffer Solution (50X), Concentrate 20ml. If using full bottle add 980ml double de-ionised water and mix. Once diluted the wash buffer solution is stable for 30days when stored at 2-8oC. (Detergent Tween-20)
FT05-010 (1x8ml)	**STOP SOLUTION:** Colorless liquid in a clear vial with red screw cap. Diluted HCL acid solution. Ready to use as supplied.

FT05-011 (1x14ml)	**SUBSTRATE SOLUTION:** Amber vial. Clear solution, ready-to-use.
FT05-012 (1x8ml)	**CONJUGATE CONC.:** Amber vial Ready – To – Use
FT05-014 (2x25ml)	**DILUTION BUFFER (1X)** Plastic bottle, Clear Solution. Ready – to – use.
1x	**PLATE COVER** To cover the strips during the incubation.
1x	**IFU** (Instruction Manual)

Materials required but not provided

- Micro-pipettes with disposable plastic tips (10-200 and 200-1000 µL)
- Multi-channel pipette (10-250 µL) or stepper pipette with plastic tips (10-250 µL)
- Microtiter plate washer (optional) or wash bottles
- Microtiter plate reader (wave length 450 nm)
- Blender/grinding pestle and mortar
- Roller/shaker
- Vortex
- pH meter
- Sample Evaporator
- Centrifuge, capable of spinning at 3,000 X g
- Timer
- Tape or Parafilm
- Distilled water
- 0.01M and 1M HCL
- 1M and 2M NaOH
- pH strips
- Isobutanol AnalR grade
- !M Sodium Carbonate buffer

Safety Precautions

The standard solutions in this test kit contain small amounts of Clenbuterol. In addition, the substrate solution contains tetramethylbenzidine and the stop solution contains diluted sulfuric acid. Avoid contact of stopping solution with skin and mucous membranes. If these reagents come in contact with the skin, wash with water.

Handling and Storage Instructions

The Beta Agonist ELISA should to be stored in the refrigerator (2–8°C). The solutions must be allowed to reach room temperature (20-25°C) before use. Reagents may be used until the expiration date on the box. Reseal the unused strips of the microtiter plate in the bag together with the desiccant bag provided. Do not intermix components from different lots.

Sample Preparation

Urine

1. Centrifuge the sample at 3000g for 10mins
2. Dilute supernatant 5 fold eg 100ul + 400ul dilution buffer
3. Apply the sample directly to the assay

Feed

1. Finely grind or blend a homogenous quantity of feed sample
2. Weigh out 1g and add 100ml of 0.01M HCL
3. Vortex 2mins and roll for 10mins
4. Adjust pH of sample to between 6.5 – 8 using 1M NaOH or 1M HCL
5. Remove 1ml aliquot
6. Centrifuge sample 3000g for 10mins
7. Apply the supernatant directly to the microtiter plate

Liver

1. Homogenise/blend liver sample
2. Weigh out 1g samples and add 1ml 0.01M HCL
3. Vortex 2mins and roll for 10mins
4. Centrifuge sample 3000g for 15mins
5. Remove 250ul of the supernatant into a clean Eppendorf 1.5ml tube
6. Adjust pH to 7 by adding 5ul 2M NaOH
7. Centrifuge sample 3000g for 10mins
8. Dilute supernatant 4 fold eg 100ul + 300ul dilution buffer
9. Apply the sample to the microtiter plate

Please note sample should be used in assay directly after final centrifugation

Muscle

1. Homogenise/blend muscle sample
2. Weigh out 1g sample and add 4ml 0.01M HCL
3. Vortex 2mins and roll for 10mins
4. Centrifuge sample 3000g for 15mins
5. Remove 2ml of the supernatant and add 0.25ml 1M carbonate buffer (ph 9.8)
6. Adjust pH to 9.8 by adding 1M NaOH
7. Add 4ml of isobutanol and vortex for 1min.
8. Centrifuge sample 3000g for 10mins
9. Remove 2ml of upper organic layer
10. Evaporate to dryness under stream of air or nitrogen at 50°C
11. Resuspend dried sample in 500ul deionized water
12. Apply the sample to the microtiter plate

Preparation of Reagents

Micro-pipetting equipment and pipette tips for pipetting the standards and the samples are necessary. We recommend using a multi-channel pipette or a stepping pipette for adding the conjugate, antibody, substrate and stop solutions in order to equalize the incubations periods of the solutions on the entire microtiter plate. Please do not mix any reagents from various kit lot numbers.

1. Adjust the microtiter plate and the reagents to room temperature before use.
2. Remove the number of microtiter plate strips required from the foil bag. The remaining strips are stored in the foil bag with desiccant and zip-locked closed. Store the remaining kit in the refrigerator (2-8°C).
3. The standard solutions, enzyme conjugate, dilution buffer, substrate and stop solutions are ready to use and do not require any further dilutions.
4. Dilute the wash buffer concentrate at a ratio of 1:50. If using the entire bottle (20 mL), add to 980 mL of deionized or distilled water. Once diluted the solution is stable for 30days when stored at 2-8°C.
5. The stop solution should be handled with care as it contains diluted H_2SO_4.

Assay Procedure

1. Add 50 µL of the standard solutions or samples (sample extracts) into the wells of the test strips according to the working scheme given. We recommend using duplicates or triplicates.
2. Add 50 µL of enzyme conjugate solution to the individual wells successively using a multi-channel pipette or a stepping pipette. Tap or swirl to mix contents.
3. Incubate the strips for 60 minutes at room temperature.
4. After incubation, remove the covering and vigorously shake the contents of these wells into a sink. Wash the strips four times using the 1X washing buffer solution (wash bottle can be used) Use at least a volume of 250 µL of washing buffer for each well and tap the plate for 10 seconds with every wash. Remaining buffer in the wells should be removed by patting the plate dry on a stack of paper towels.
5. Add 100 µL of substrate (color) solution to the wells. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for about 30 seconds. Incubate the strips for 20 minutes at room temperature. Protect the strips from direct sunlight.
6. Add 50 µL of stop solution to the wells in the same sequence as for the substrate solution.
7. Read the absorbance at 450 nm using a microplate ELISA photometer within 15 minutes after the addition of the stopping solution.

Recommended working template

The microtiter plate consists of 12 strips of 8 wells, which can be used individually for the test. The standards must be run with each test. Never use the values of standards which have been determined in a test performed previously.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Std 1	Std 1	Smp 2	Smp 2								
B	Std 2	Std 2	Smp 3	Smp 3								
C	Std 3	Std 3										
D	Std 4	Std 4										
E	Std 5	Std 5										
F	Std 6	Std 6										
G	QC	QC										
H	Smp 1	Smp 1										

Std 1-Std 6: Standards
0; 0.037; 0.075; 0.15; 0.6; 2.5ppb

Quality Control

Samp1, Samp2, etc.: Samples

Calculation of results

1. Calculate the average optical density (OD 450 nm) for each set of reference standards or samples.
2. Construct a standard curve by plotting the mean optical density obtained for each reference standard against its concentration in ng/mL on semi-log graph paper with the optical density on the vertical (y) axis and the concentration on the horizontal (x) axis.
3. Using the mean optical density value for each sample, determine the corresponding concentration of clenbuterol in ng/mL from the standard curve. Depending on experience and/or the availability of computer capability, other methods of data reduction may be employed.
4. The diluted samples must be further converted by the appropriate sample dilution factor.

Results Evaluation

URINE

The ELISA result should be multiplied by a factor of 5 to obtain the final Beta Agonist concentration in the sample.

FEED

The ELISA result should be multiplied by a factor of 100 to obtain the final Beta Agonist concentration in the sample.

LIVER

The ELISA result should be multiplied by a factor of 8 to obtain the final Beta Agonist concentration in the sample

MUSCLE

The ELISA result should be multiplied by a factor of 2.5 to obtain the final Beta Agonist concentration in the sample