Biorex food diagnostics

Trenbolone ELISA

PRODUCT CODE: BXEFT15A

QUALITY MANAGEMENT SYSTEM
ISO 13485 CERTIFIED COMPANY

BXEFT15A
96 TESTS (12x8 STRIP WELLS)
STORE AT 2-8°C
INSTRUCTIONS FOR USE
FOR USE IN THE ANALYSIS OF FOOD AND VETERINARY 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

Trenbolone ELISA

Introduction

Enzyme-Linked Immunosorbent Assay for the Determination of Trenbolone in muscle and urines.

Importance of Trenbolone determination

Trenbolone, also known as trienolone or trienbolone, is an anabolic-androgenic steroid (AAS) of the 19-nortestosterone group. It is a synthetic xenobiotic growth promoter used in veterinary medicine in livestock to increase muscle growth and appetite, feed efficiency, and mineral absorption in cattle.

Analysing the residue level of the anabolic agents in different meat and meat products is important as they may pose a potential risk to public health.

The use of hormonal growth promoters in food-producing animals has provoked many concerns on their human health impacts.

Recent results of risk assessments on hormonal substances including Trenbolone, indicate that for synthetic hormone-like substances, ADIs and MRLs need to be provided for the protection of human health.

Samples

Urine and muscle

Sample Preparation

Solid Phase Extraction

Assay Time

105mins

Limit of detection

Urine 0.25ppb
Muscle 0.1ppb

Specificity

Compound	% Specificity
17-β-Trenbolone	100 %
17-β-Trenbolone Glucuronide	78 %
17-a-Trenbolone	58 %
19-Nortestosterone	11 %
Estrone	0.3%
17-Methyltestosterone	< 0.05%
17-β-Estradiol	< 0.05%
Testosterone	<0.05%
Estriol	< 0.05%

Test Principle

This test kit is a competitive immunoassay. The assay is performed in microwells which have been pre-coated with anti-rabbit IgG. Trenbolone standards, samples, enzyme conjugate Trenbolone-Peroxidase, and anti-Trenbolone antibodies are added into the microwells. During the incubation, free and conjugate Trenbolone compete for the anti-Trenbolone antibodies binding points. At the same time, the anti-Trenbolone antibodies are also bound by the immobilised goat IgG. After allowing these reactions to proceed, the unbound material is removed in a washing step. The bound enzyme activity is revealed by adding a fixed amount of colorimetric substrate which develops a blue coloration. The colour development is inversely proportional to the Trenbolone concentration in the sample, which is determined by reading off a calibration curve derived from standards of known concentration.

Reagents Provided

The kit contains reagents for 96 determinations. They have to be stored at 2-8°C. Expiry data are found on the labels of the bottles and the outer package.

Code / Size	Component / Description
FT15 -001 **FT15 -002** **FT15 -003** **FT15 -004** **FT15 -005** **FT15 -006** (6 vials with 1.5mL each)	STANDARD A – 0 ng/ml STANDARD B – 0.025 ng/ml STANDARD C –0.1 ng/ml STANDARD D –0.3 ng/ml STANDARD E – 1 ng/ml STANDARD F –3 ng/ml Ready-to-use
FT15 -008 (1x96 wells) 8×12/12×8 well per plate	**MICROTITER PLATE** consisting of 12 strips with 8 breakable wells each, coated with anti-rabbit IgG.
FT15-009 (2x25ml) ***Requires dilution!***	**WASH BUFFER:** Wash Buffer Solution (10X). Dilute 1:10 with distilled water prior to use.
FT15 -010 (1x8ml)	**STOP SOLUTION:** Colourless liquid in a clear vial with red screw cap. Diluted acid solution. Ready to use as supplied.

FT15 -012 (1x8ml)	**CONJUGATE:** Black plastic vial, Red solution, Ready-to-use.
FT15 -013 (1x8ml)	**ANTIBODY SOLUTION:** Plastic Bottle, Blue solution, Ready -to-use.
FT15-014 (2x25ml)	**DILUTION BUFFER:** Plastic Bottle, Orange solution, Ready-to-use.
FT15 -022 (1x14ml)	**SUBSTRATE** Clear solution, ready-to-use.
1x	**PLATE COVER** To cover the strips during the incubation.
1x	**IFU** (Instruction Manual)

Materials required but not provided

Instrumentation

- 20, 50, 100, 500 and 1000 µL-micropipettes
- 10,000 µL-pipette or glass pipette
- ELISA reader (450 nm)
- pH meter
- C18 reverse phase columns (J.T.Baker 200 mg cat.7020-02)
- *Helix pomatia* β-glucuronidase (Sigma cat.n. G7017, 100,000 units/ml).
- 37°C incubator
- Vacuum centrifuge or nitrogen/air flow evaporator system

Reagents

- Distilled water
- Methanol, reagent grade
- Terbuthylmethylether
- 50mM sodium acetate buffer pH 4.8 (for urine)
- tris buffer (20mM Tris/HCl buffer pH 8.5) (for urine)
- 50 and 100 µl precision micropipettes
- 30 - 300 µl multichannel micropipette

Safety Precautions

1. Do not smoke or eat or drink or pipet by mouth in the laboratory.
2. Wear disposable gloves whenever handling patient specimens.
3. Avoid contact of developing solution and stop solution with skin and mucosa (possible irritation, burn or toxicity hazard). In case of contact, rinse the affected zone with plenty of water.
4. Handling and disposal of chemical products must be done according to good laboratory practices (GLP).

Handling and Storage Instructions

Full compliance of the following good laboratory practices (GLP) will determine the reliability of the results:

1. Prior to beginning the assay procedure, bring all reagents to room temperature (20-25°C).
2. All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
3. Once the assay has been started, all subsequent steps should be completed without interruption and within the recommended time limits.
4. Replace caps in all the reagents immediately after use. Do not interchange vial stoppers.
5. Use a separate disposable tip for each specimen to prevent cross-contamination.
6. All specimens and standards should be run at the same time, so that all conditions of testing are the same.
7. Do not mix components from different batches.
8. Do not use reagents after expiration date.
9. Check both precision and accuracy of the laboratory equipment used during the procedure (micropipettes, ELISA reader etc.).

Preparation of working solutions

1. Standard solutions: ready to use
2. Anti-Trenbolone antibody: ready to use
3. Wash Buffer Solution (10X): Concentrate 50 ml. If using full bottle add 450 ml double de-ionised water and mix. Once diluted the wash buffer solution is stable for 2 weeks when stored at 2-8°C. (Detergent Tween-20)
4. Enzyme conjugate: ready to use
5. Developing solution: ready-to-use. This solution is light sensitive and so should be kept in the dark.
6. Stop solution: ready to use. Contains acid: handle with care; in case of contact was thoroughly with tap water

Sample Preparation

Urine

1. Centrifuge at 3000g for 5 min
1. To 0.5ml of the supernatant add 2.5ml sodium acetate buffer (50mM pH 4.8). Adjust pH if required.
2. Add 10ul of *Helix pomatia* β-glucuronidase to sample and mix. Ensure pH remains at 4.8-5.0. Incubate 2hrs at 37°C or 16hrs overnight at room temperature (20-25˚C)

Urine SPE Clean up

1. Equilibrate columns by adding
   a. 3 ml Methanol 100%.
   b. 2 ml of (20:80) methanol/Tris HCl 20 mM pH 8.5. (1drop/sec)

Please note columns should not be allowed to dry out before adding the sample. If this occurs repeat steps a and b.

2. Add the deconjugated urine sample (2ml) after incubation directly to the column (gravity flow).
3. Wash the column;
   a. 2ml of (20:80) methanol/Tris HCl 20 mM pH 8.5
   b. 2ml of 40:60 methanol/water
   c. Using a vacuum, dry the column. This should take approx. 2 mins
4. Elute by adding 1 ml 80:20 methanol/water (gravity flow) into a clean glass test tube. It is advisable to use only new glass tubes or treated with sulfochromic mix.
5. Dry the eluate in a vacuum centrifuge or with nitrogen/air flow placing the tubes at 60°C
6. Resuspend the dried sample with 500 µl of dilution buffer thoroughly mixing with vortex for 2 minutes
7. Apply sample to microtiter plate.

Muscle

1. Add 1ml of 67mM phosphate buffer pH 7.2 + 67mM NaCL to 1g muscle in a glass test tube. Vortex for 1 min to mix
2. Add 5ml Terbuthylmethylether to the glass test tube. Vortex for 2 mins on gentle speed and rotate for 20mins.
3. Centrifuge at 3000g for 10 mins
4. Transfer 4ml of the supernatant to a fresh glass test tube. Vortex for 30 secs

5. Repeat steps 2-4 but add the 4ml supernatant to the test tube to which 4ml have already been added in step 4 above
6. Evaporate the supernatants with nitrogen/air flow at 40˚C
7. Resuspend the dried sample in 0.8ml of methanol/water (80/20)
8. Dilute sample in 2ml Buffer 20mM phosphate buffer pH 7.2 + 20mM NaCl
9. Cool samples
10. Centrifuge at 3000g for 10mins

Muscle SPE Clean up

1. Equilibrate columns by adding
   a. 3 ml Methanol 100%.
   b. 2 ml of 20mM phosphate buffer pH 7.2 + 20mM NaCl

Please note columns should not be allowed to dry out before adding the sample. If this occurs repeat steps a and b.

2. Add 2ml of the sample directly to the column (gravity flow).
3. Wash the column;
   b. 2ml of 40:60 methanol/water
   c. Using a vacuum, dry the column. This should take approx. 3 mins
4. Elute by adding 1 ml 80:20 methanol/water (gravity flow) into a clean glass test tube. It is advisable to use only new glass tubes or treated with sulfochromic mix.
5. Dry the eluate in a vacuum centrifuge or with nitrogen/air flow placing the tubes at 60°C
6. Resuspend the dried sample with 570 µl of dilution buffer (1X) thoroughly mixing with vortex for 2 minutes
7. Apply sample to microtiter plate.

Assay Procedure

1. Prepare samples as described above.
2. Pipet:

- 50 µL standards and prepared samples in duplicate into the appropriate wells
- 50 µL conjugate into each well of the microtiter plate.
- 50 µL antibody solution into each well of the microtiter plate.

3. Cover the wells with the microtiter plate cover provided and mix the contents by moving the strip holder in a circular motion on the benchtop for 30 seconds or by tapping side of plate. Be careful not to spill contents.
4. Incubate for 90 minutes at room temperature.
5. After incubation, remove the covering and vigorously shake the contents of these wells into a sink. Wash the strips using a wash bottle five times using the 1X wash buffer solution. Use 300 µL of washing buffer for each well and tap the plate for 10 seconds for every wash. On the last wash the remaining buffer in the wells should be removed by patting the plate dry on tissue paper/lint free paper
6. Pipet 100 µL of substrate solution into each well and tap for 10 seconds to mix.
7. Allow the reaction to develop in the dark for 15 minutes incubate at room temperature.
8. Stop enzyme reaction by adding 50 µL of stop solution into each well.
9. After thorough mixing, measure absorbance at 450 nm (reference wavelength 630 nm if available), using an appropriate reader. A 4 parameter curve fit is recommended. The colour is stable for 30 minutes

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 A	Std A	Smp2	Smp2								
B	Std B	Std B										
C	Std C	Std C										
D	Std D	Std D										
E	Std E	Std E										
F	Std F	Std F										
G	QC	QC										
H	Smp1	Smp1										

Std A-Std F: Standards
0 ng/ml, 0.025 ng/ml, 0.1 ng/ml, 0.3 ng/ml, 1 ng/ml, 3 ng/ml
QC: Quality Control
-Smp1, Smp2, 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. Use the mean optical density value for each sample, determine the corresponding concentration of Trenbolone in ng/mL from the standard curve. Depending on experience and/or the availability of software, other methods of data reduction may be employed. A 4 parameter curve fit is recommended.
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 10 to obtain the final Trenbolone concentration in the sample.

MUSCLE

The ELISA result should be multiplied by a factor of 1 to obtain the final Trenbolone concentration in the sample.