UC Biodevices

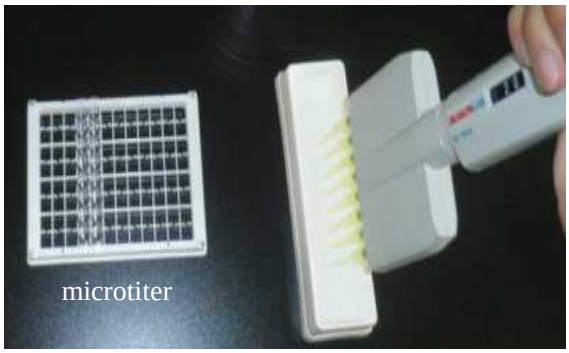
ELISA Kit for Detection of ß-Agonists

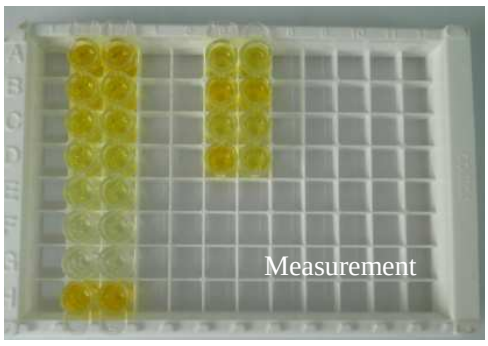
(Product Number: 5501E209)

（ß-Agonists can cause life threat to human）

INSTRUCTION MANUAL

(v. 1.00)





1. Introduction

ß-Agonists, such as clenbuterol or salbutamol, are synthetic derivatives of the naturally occurring catecholamines. It has been known that ß-agonists are suitable for use as performance improvers within the field of livestock production; in particular, the meat/fat ratio in fattened animals can be improved or the growth may be accelerated. However, such compounds have not been approved in the EU for use as fattening adjuvants. In addition to lipolytic and anabolic effects, ß-agonists have relaxing effects on non-striated musculature, allowing them to be used as antiasthmatic and tocolytic agents. Actually, ß-agonists residues, after use in illegal practice, may lead to a risk for consumers, and there is a prohibition of ß-agonists use in food production.

This kit is based on indirect-competitive ELISA technology. The microtiter wells are coated with coupling antigen. Residue in the sample competes with the antigen coated on the microtiter plate for the antibody. After the addition of enzyme labeled anti-antibody, TMB substrate is used to show the color. The addition of the stopping solution leads to a color change from blue to yellow. The measurement is performed photometrically at 450 nm. The absorption is inversely proportional to the ß-agonists concentration in the sample.

Assay Sensitivity, Precision, Accuracy

1) Sensitivity: 0.1ppb
2) Precision：<20%
3) Accuracy：80-120％ recovery rate

2. Kit Contents

1) Microtiter plate (8 wells×12) precoated with antigen.
2) 7×Standard solutions (1ml/each):0ng/ml, 0.1ng/ml, 0.3ng/ml, 1.0ng/ml, 3.0ng/ml, 9.0ng/ml, 27.0ng/ml

   Controls provided: 0ppb, 0.1ppb, 0.3ppb, 1.0ppb, 3.0ppb, 9.0ppb and 27.0ppb
3) Antibody (50×) (0.2ml)
4) Enzyme Conjugate(60×) (0.2ml)
5) washing buffer(10×) (50ml)
6) Antibody solution (15ml)

7) Diluent buffer A (20ml)
8) Diluent buffer B (40ml)
9) Diluent buffer C (40ml)
10) TMB (18ml)
11) Stopping solution (7ml)
12) Instruction Manual

Materials and reagents required but not provided:

1) variable 20 µl - 200 µl and 200 - 1000 µl micropipettes
2) microtitre plate reader with 450nm filter.
3) Acetonitrile - ACS grade or better.
4) n-Hexane
5) Shaker
6) 50mL centrifuge tube、funnel and paper filter
7) Thermostat
8) 100ml graduated cylinder.
9) Centrifuge（4000r/min）and vortex mixer
10) Timer

3. Preparation of Working Solutions

- S**tandard solutions**: ready to use.
- **50×Antibody**: dilute 50× with Diluent buffer A(1+49).
- **60× Enzyme Conjugate**: dilute 60× with Antibody solution (1+59).
- **washing buffer**: dilute 10× with distilled water (1+9).
- **TMB**: ready to use.
- **stopping solution**: ready to use.

4. Preparation of Samples

Urine

a. centrifugati :10 min / 4000 g

b. Dilute 100µl of the Supernatant with 500 of Diluent buffer B

c. use 50µl per well in the assay

Meat

a. transfer 4 g of the homogenized sample into a centrifugal screw cap vial, add 8ml acetonitrile and shake for 30 min (with up-side-down shaker)

b. centrifuge: 10 min/4000g / room temperature

c. transfer 4 ml of the acetonitrile solution into a new centrifugal vial and evaporate to dryness at 50℃

d. dissolve the residue in 2 ml n-hexane, add 1 ml Diluent buffer A and vortex for 30 sec

e. centrifuge: 10 min/4000 g/room temperature

f. transfer the aqueous (lower) phase into a new vial and Evaporate to dryness under a nitrogen stream at approximately 50℃ for 10 min.

g. Dilute 100µl of the filtrate with 500 of Diluent buffer C

h. use 50µl per well in the assay

5. Immunoassay Procedure

a) Allow reagents, microwells, and sample extracts to reach room temperature prior to running the test.

b) Insert a sufficient number of wells into the microwell holder for all standards and samples to be tested.

c) Using a new pipette tip for each standard and sample, pipette 50µl of standards and prepared sample to separate wells.

d) Add 50µl of enzyme conjugate into each well.

e) Add 50µl of anti- ß-agonists antibody into each well.

f) Incubate for 20 minutes at room temperature (37℃)

g) Dump the contents of the wells. Turn the wells upside down and tap out on a paper towel until the remaining liquid has been removed.

h) Using a wash bottle, fill each well with washing buffer. Empty the wells again and remove all remaining liquid. Repeat this step 2 times (total of 4 washes).

i) Add 150µl of TMB to each well. Incubate for 10 minutes at room temperature (37℃). Cover the wells with a paper towel to protect them from light sources.

j) Add 50µl of stop solution to each well.

k) Read results using a microwell reader with a 450 nm Thermo Labsystems.

6. Calculation

Divide the mean absorbance value of standards and samples (B) by the mean absorbance value of the Maximum Binding (Bo) and multiply by 100. Maximum binding is thus made equal to 100% and the absorbance values are quoted in percentages:

$$\frac{\text{absorbance standard (or sample)}}{\text{absorbance maximum binding}} \times 100 = B/Bo\ \%$$

7. Results

Since the samples have been diluted prior to assay, the concentrations in ng/ml read from the standard curve must be multiplied by the respective dilution factor to obtain the effective ß-agonists concentration in samples expressed in ppb (ng/ml or ng/g). The dilution factors are 5 for urine, 2.5 for meat.

8. Cautions

1) If ambient temperature is lower than 20℃ or the temperature of the reagents and standard solutions is less than the ambient temperature (e.g., 20- 25℃） when used, the testing results may have negative bias (lower than it should be).
2) If it takes too long time to dry the washed microtiter during the washing steps, the results may demonstrate nonlinear calibration plot and poor repeatability, thus once the microtiter is dried after the washing steps, the following step should be immediately implemented to prevent the undesirable occurrence.
3) The standard solutions and chromogenic solution contain light-sensitive substances, thus the solutions should not be exposed under direct light.
4) To gain excellent results, any solutions should be thoroughly mixed before added on microtiter and the microtiter should be completely cleaned in between each step.
5) The stopping solution is a very strong acidic solution, so users should avoid the solution directly

contacting with skin.

6) Neither use expired reagents, nor dilutes reagents or employs reagents produced by varied companies; otherwise, only gain less sensitive results.

7) Do not interchange individual reagents between kits of different lot numbers.

8) The kit should be stored at 2-8℃ but not be frozen. Place any unused microwells to their original foil bag and reseal them together with the desiccant provided.

9. Contact Information

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August, 2011

50010015 V1.00