



# Cimaterol ELISA Kit

10/19

(Catalog # E4771-100; 96 assays, Storage at 4°C)

#### I. Introduction:

Cimaterol is a chemically stable nonselective agonist  $\beta1$ -,  $\beta2$ -, and  $\beta3$ -adrenoceptors. Cimaterol is used in sport as a stimulant and a fat burner that assists bodybuilders and strength athletes to get rid of body fats. Cimaterol has been used in farmed animals (swine, fowl, etc.) to increase carcass mass and to alter muscle and fat deposition. BioVision's Cimaterol ELISA Kit is based on the Competitive ELISA method. It can detect Cimaterol in Tissue, Urine and Feed samples. The microtiter plate provided in the kit has been pre-coated with Cimaterol. During the reaction, Cimaterol in the samples or standard competes with coated Cimaterol for anti- Cimaterol antibody. Then Horseradish Peroxidase (HRP) conjugate is added to each microtiter plate well, and TMB substrate is added for color development. There is a negative correlation between the OD value of samples and the concentration of Cimaterol. The concentration of Cimaterol in the samples can be calculated by comparing the OD of the samples to the standard curve.

## II. Applications:

In vitro, quantitative determination of Cimaterol

Detection Range: Urine - 0.3 ppb, Tissue (low detection limit method) - 1.2 ppb, Tissue (high detection limit method) - 0.3 ppb, Feed- 3

Sensitivity: 0.3 ppb (ng/mL)

Sample recovery rate: Urine - 95%±10%, Tissue, Feed- 85%±15%.

Cross-reactivity: Clenbuterol - 100%, Arubendol - <1%, Mabuterol - <1%, Brombuterol - <1%, Salbutamol - <1%, Ractopamine- <1%.

#### III. Sample Type:

Tissue, Urine, Feed

#### IV. Kit Contents:

Components	E4771-100	Part Number
Micro ELISA Plate	96 wells	E4771-100-1
Standard	6 X 1 ml	E4771-100-2
HRP Conjugate	5.5 ml	E4771-100-3
Antibody Working Solution	5.5 ml	E4771-100-4
Substrate Reagent A	6 ml	E4771-100-5
Substrate Reagent B	6 ml	E4771-100-6
Stop Solution	6 ml	E4771-100-7
Wash Buffer (20X)	40 ml	E4771-100-8
Reconstitution Buffer (10X)	50 ml	E4771-100-9
Plate Sealer	3	E4771-100-10

## V. User Supplied Reagents and Equipment:

- · Microplate reader capable of measuring absorbance at 450 nm
- HCl, Acetonitrile, NaOH
- Clean Eppendorf tubes for preparing standards or sample dilutions

# VI. Storage and Handling:

Store at 4°C.

# VII. Reagent and Sample Preparation:

Bring all reagents to room temperature before use. Before using the kit, spin tubes and bring down all components to the bottom of tubes.

- Wash Buffer (20X): Dilute 20X Concentrated Wash Buffer to 1X with deionized water.
- Reconstitution Buffer (10X): Dilute 10X Reconstitution Buffer with deionized water. Mix 10x Reconstitution Buffer (V): Deionized water (V) =1:9). The Reconstitution buffer can be store at 4°C for a month.

## · Standard:

Standard	S1	S2	S3	S4	S5	S6
Concentration (ppb)	0	0.3	0.6	1.2	2.4	4.8

#### Solution preparation

- 0.1 M HCI: Dilute 0.86 mL Concentrated HCl with deionized water to 100 ml.
- 0.1 M NaOH: Dissolve 0.4 g NaOH with 100 ml deionized water

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• Acetonitrile 0.1 M HCl: Acetonitrile (V): 0.1 M HCl solution (V) =84:16

# VIII. Sample Preparation:

## Sample pretreatment:

## • Pretreatment of tissue (low detection limit method):

Weigh  $2\pm0.05g$  of crushed homogenate tissue sample; add 6mL of 1X Reconstitution Buffer. Mix well for 2 min, centrifuge at 4000 rpm for 10min (incubate the sample at 85°Cfor 10 min before centrifugation if there is a high-content of fat in tissue sample). Take 50  $\mu$ l of the supernatant for analysis.

Note: Sample dilution factor: 4, minimum detection limit: 1.2ppb.

# Pretreatment of tissue (high detection limit):

Weigh 2 ± 0.05 g of crushed homogenate tissue sample, add 6 ml of Acetonitrile 0.1 M HCl. Mix well for 2 min, centrifuge at 4000 rpm for 10 min at room temperature. Take 3 mL of the supernatant. Add 2 mL of 0.1 M NaOH and 6 mL of Ethyl acetate. Mix well for 2 min, centrifuge at 4000 rpm for 10 min at room temperature. Take all the supernatant and dry at 50-60°C with nitrogen evaporators or water bath. Add 1 mL of 1X Reconstitution Buffer and oscillate for 30 sec. Take 5 0 µl for analysis.

Note: Sample dilution factor: 1, minimum detection limit: 0. 3 ppb

#### • Pretreatment ofswine urine:

Take 50 µl of clear urine sample for analysis directly (if the urine sample is turbid, it should be filtered or centrifuged at 4000 rpm for 5 min until the urine sample become clear). Freeze the samples for later use.

Note: Sample dilution factor: 1, minimum detection limit: 0.3ppb

#### Pretreatment of feed sample:

Weigh 1  $\pm$  0.05 g of homogenate feed sample, add 10 mL of Methanol and 5 g of Na<sub>2</sub>SO<sub>4</sub>. Mix well for 2 min, centrifuge at 4000 rpm for 10 min at room temperature. Take 1 mL of the supernatant and dry with nitrogen evaporators/water bath at 50-60 °C. Add 1 ml of Reconstitution Buffer (Solution to dissolve the remaining dry material. Then add 1 mL of N hexane and mix for 30 sec. Centrifuge for 5 min at 4 000 rpm at room temperature. Take 50  $\mu$ l of the lower layer liquid for analysis.

Note: Sample dilution factor: 10, minimum detection limit: 3 ppb.

## IX. Assay Protocol:

**Note:** Bring all reagents and samples to room temperature 30 minutes prior to the assay. It is recommended that all standards and samples be run at least in duplicate. A standard curve must be run with each assay.

- 1. Add 50 µl of each standard or samples into appropriate wells.
- 2. Add 50 µl of HRP Conjugate to each well and then add 50 µl of Antibody Working Solution. Cover the plate with the sealer provided in the kit. Gently mix and incubate for 30 min. at 25°C. **Note:** solutions should be added to the bottom of the micro ELISA plate well, avoid touching the inside wall and bubble formation as much as possible.
- 3. Aspirate the solution from each well add 300 µl of 1x wash buffer to each well. Leave it for 30 sec, aspirate the solution from each well and pat it dry against clean absorbent paper. Repeat this wash step 5 times.
  - Note: a microplate washer can be used in this step and other wash steps.
- 4. Add 50 µl of **Substrate Reagent A** to each well and then add 50 µl of **Substrate Reagent B**. Cover with a new plate sealer. Incubate for about 15 min at 25°C. Protect the plate from light. Note: the reaction time can be shortened or extended according to the actual color change, but not more than 30 min.
- 5. Add 50 µl of **Stop Solution** to each well. Note: adding the stop solution should be done in the same order as the substrate solution.
- 6. Read the absorbance in micro plate reader set to 450 nm reference wavelength 630 nm. This step should be performed within 5 min after stop reaction.

# X. Calculation:

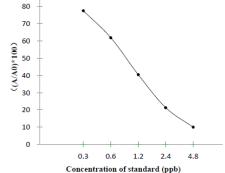
Create a standard curve by plotting the absorbance percentage of each standard on the y-axis against the log concentration on the x-axis to

draw a semi logarithmic plot. Add average absorbance value of sample to standard curve to get corresponding concentration. If samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor.

## Absorbance (%)=A/A<sub>0</sub> ×100%

A: Average absorbance of standard or sample

A<sub>0</sub>: Average absorbance of 0 ppb Standard



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Typical standard curve and data is provided below for reference only. A standard curve must be run with each assay

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Concentration of standard (ppb)	OD-1	OD-2	Average OD
0	2.7721	2.7414	2.7568
0.3	2.1215	2.1519	2.1367
0.6	1.7033	1.7071	1.7052
1.2	1.1203	1.1087	1.1145
2.4	0.5717	0.6012	0.5865
4.8	0.2651	0.2844	0.2748

# **XI. RELATED PRODUCTS:**

- Enrofloxacin (ENR) ELISA Kit (E4277)
- Chloramphenicol (CAP) ELISA Kit (K4230)
- Gentamicin ELISA Kit (K4206)
- Ciprofloxacin (Cipro) ELISA Kit (E4365)

FOR RESEARCH USE ONLY! Not to be used on humans.