Technical specifications

Sensitivity: 0.1 ppb

Detection limit

Tissue	0.1 ppb
Urine, serum	0.1 ppb
Feed	10 ppb

Recovery rate

Urine, serum	90±10%
Tissue	80±10%
Feed	80±15%

Cross-reaction rate

Clenbuterol	100%
Mabuterol	95%
Brombuterol	115%
Salbutamal	< 11%
Terbutalin	< 7%
Ractopamine	< 1%

Antibiotics ELISA kits available from ADI:

http://4adi.com/commerce/catalog/spcategory.jsp?category_id=2739

DE-100030	Salbutamal ELISA kit, For Urine, Tissue, Feed, Animal Tissue, Aquatic, Honey, Intestine,, 96 tests
DE-100040	Chloramphenicol ELISA kit, 96 tests (For Animal Tissue, Aquatic, Honey, Intestine, Urine, Egg, Milk, Serum)
DE-100050	Florfenicol ELISA kit (For Animal Tissue, Aquatic, Honey), 96 tests
DE-100060	Nitrofuran (AMOZ) ELISA kit (For Fish, Shrimp, Honey, Chicken/Liver), 96 tests
DE-100070	Nitrofuran (AHD) ELISA kit, (For Fish, Shrimp, Honey, Chicken/Liver), 96 tests
DE-100075	Nitrofuran (SEM) ELISA kit (Honey, Fish, Shrimp, Chicken/Liver, Fish/Shrimp), 96 tests
DE-100080	Nitrofuran (AOZ) ELISA kit (For Fish, Shrimp, Honey, Chicken/Liver), 96 tests
DE-100090	Sulfonamides Residues (SAs) ELISA kit, (For Chicken/Liver, Pork/Liver, Honey/Egg, Serum/Urine, Milk), 96 tests
DE-100100	Sulfamethazine (SM2) ELISA kit, 96 tests (For Chicken/Liver, Pork/Liver, Honey/Egg, Serum/Urine, Milk)
DE-100110	Sulfamethoxydiazine (SMD) ELISA kit, (For Chicken/Liver, Pork/Liver, Honey/Egg, Serum/Urine), 96 tests
DE-100120	Quinolones (QNS) ELISA kit (For Pork/Liver, Chicken/Liver, Shrimp, Fish, Serum, Honey), 96 tests
DE-100130	Enrofloxacin ELISA kit (For Pork/Liver, Chicken/Liver, Shrimp, Fish, Serum, Honey), 96 tests
DE-100140	Ampicillin ELISA kit, (For Pork/Liver, Chicken, Duck, Shrimp, Fish, Honey, Milk), 96 tests
DE-100150	Benzyl Penicillin ELISA kit, (For Pork/Liver, Chicken, Duck, Shrimp, Fish, Honey, Milk), 96 tests
DE-100160	Tylosin ELISA kit (For Meat, Liver, Honey, Egg), 96 tests
DE-100170	Trenbolone ELISA kit (For Animal Tissue, Aquatic, Urine), 96 tests
DE-100180	Diazepam ELISA kit (For Tissue, Urine, Feed), 96 tests
DE-100190	Diethylstilbestrol (DES) ELISA kit (Fish, Shrimp, Liver, Meat, Feed, Urine), 96 tests
DE-100200	Gentamicin ELISA kit (Chicken/Liver), 96 tests
DE-100210	Streptomycin ELISA kit, 96 tests (Chicken/Liver, Honey, Milk)
DE-100230	Olaquindox ELISA kit (Tissue) 96 tests
DE-100240	Sulfaquin-oxaline ELISA kit, (For Pork/Liver, Honey/Egg, Serum/Urine, Milk), 96 tests

See Details at the web site or Contact ADI

Instruction Manual No. M-DE-100010

Clenbuterol ELISA KIT

Cat. #DE-100010

For Qualitative and Quantitative Determination
of Clenbuterol in pork liver, serum, urine and feed.

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LIFE TECH Life Technologies™
Your Molecular & Cell Technology Partner

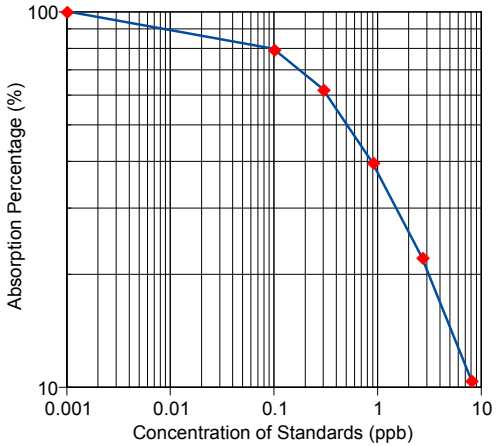
Clenbuterol ELISA KIT Cat. #DE-100010

Kit Components, 96 tests	Cat #
Micro-well coated strip plate (12 strips with 8 removable wells each)	DE-100011
6x standard solution (1ml each): 0.0 ppb, 0.1 ppb, 0.3 ppb, 0.9 ppb, 2.7 ppb, 8.1 ppb	DE-100012
Enzyme conjugate (7ml)	DE-100013
Antibody working solution (10ml)	DE-100014
Substrate A solution (7ml)	DE-SSA
Substrate B solution (7ml)	DE-SSB
Stop solution (7ml)	DE-ST
Instruction Manual	DE-WB

INTRODUCTION

Clenbuterol is a drug used by people who have breathing disorders. It is usually offered as Clenbuterol hydrochloride which is a salt form. It helps people with asthma to decongest and dilate the bronchi and bronchioles. Its chemical formula is $C_{12}H_{18}Cl_2N_2O$ and its molecular weight is 277.19 g/mol. Clenbuterol is a Beta-2 agonist, which is a chemical with similar effects as epinephrine, a type of adrenaline, but it lasts for a longer time and is more powerful than other drugs. The main difference between clenbuterol and adrenaline is that clenbuterol stimulates only the target receptor Beta-2, on the other hand, adrenaline causes a variety of physiologic responses. Beta-2 agonist stimulates the closing of calcium channel helping adenylate cyclase. Adenylate cyclase is an enzyme that catalyzes the formation of adenosine triphosphate (ATP) to cyclic adenosine monophosphate (cAMP) and pyrophosphate. This is a very important passage since ATP is the main energy storage and transfer molecule in the cell.

Clenbuterol promotes muscle growth, increases the aerobic ability, stimulates the central nervous system and increases blood pressure and oxygen transportation. It also increases the metabolism rate of fat and protein which at the same time slows the body's basal metabolic rate. In some countries clenbuterol is approved and used under prescription as a bronchodilator for people with asthma. It is an illegal drug in the United States, it is legalized to use in race horses under a threshold limit. It is also used as a bronchodilator in horses as a treatment for allergic reactions in the respiratory tract. It is commonly known as Ventipulmin. Clenbuterol is banned from use in animal's food production, since its residues can affect human. Clenbuterol is not a hormone but it is a drug that promotes growth. Its metabolite can affect lung and heart function if digested by human. However, it can easily be purchased over the internet as a dietary supplement. This drug is very common among bodybuilders who want to lower their body fat and have a slim look. Clenbuterol is rapidly absorbed and stays in the body for about 25-39 hours.



A typical assay Standard Curve (do not use this for calculating sample values)

CALCULATION OF RESULTS

There are two methods to judge the results: the first one is the rough judgment, while the second is the quantitative determination. Note that the OD value of the sample has a negative correlation with the clenbuterol concentration.

Qualitative determination

The concentration range (ng/mL) obtained from comparing the average OD value of the sample with that of the standard solution. Assuming that the OD value of the sample Ⅰ is 0.313, and that of the sample Ⅱ is 1.032, the OD value of standard solutions is: 1.892 for 0 ppb, 1.501 for 0.1 ppb, 1.175 for 0.30 ppb, 0.751 for 0.90 ppb, 0.421 for 2.7 ppb ,0.198 for 8.1ppb, accordingly the concentration range of the sample Ⅰ is 2.7 to 8.1 ppb, and that of the sample Ⅱ is 0.30 to 0.90 ppb.

Quantitative determination

The mean values of the absorbance values is obtained for the average OD value (B) of the sample and the standard solution divided by the OD value (B0) of the first standard solution (0 standard) and subsequently multiplied by 100%, that is,

$$\% \text{ of absorbance value} = \frac{B}{B0} \times 100\%$$

B—the average OD value of the sample or the standard solution
B0—the average OD value of the 0 ng/mL standard solution

Draw the standard curve with the absorption percentages of the standard solution and the semilogarithm values of the Clenbuterol standard solution (ng/mL) as Y- and X-axis, respectively. Read the corresponding concentration of the sample from the standard curve by incorporating its absorption percentage into the standard curve. The resulting value is subsequently multiplied by the corresponding dilution fold, finally obtaining the clenbuterol concentration in the sample. Using the professional analyzing software of this kit will be more convenient for the accurate and rapid analysis of a large amount of samples. (Please contact us for this software).

4 Coloration: add 50 µL of substrate A solution and 50 µL B solution into each well. Mix gently by shaking the plate manually, and incubate at 25 ℃ for 15 min in the dark for coloration.

5 Determination: add 50 µL of the stop solution into each well. Mix gently by shaking the plate manually. Set the wavelength of the microplate reader at 450 nm to determine the OD value of every well. (Recommend to read the OD value at the dual-wavelength 450/630 nm within 5 min).

NOTES:

1. Bring all reagents and micro-well strips to the room temperature (20-25 ℃) before use, and return all reagents to 2-8 ℃ immediately after use.
2. The reproducibility of the ELISA analysis, to a large degree, depends on the consistency of plate washing. The correct operation of plate washing is the key point in the procedures of ELISA.
3. For the incubation at constant temperatures, all the samples and reagents must avoid light exposure, and each microplate should be sealed by the cover membrane.
4. The room temperature below 20 ℃ or the temperature of the reagents and the samples being not returned to the room temperature (20-25 ℃) will lead to a lower standard OD value.
5. Dryness of the microplate in the washing process will be accompanied by the situations including the non-linear standard curves and the undesirable reproducibility; So continue to next step immediately after washing.
6. The stop solution is the 2 M sulfuric acid solution, avoid contacting with the skin.
7. Do not use the kit exceeding its expiry date. The use of diluted or adulterated reagents from the kits will lead to the changes in the sensitivity and the detecting OD values. Do not exchange the reagents from the kits of different lot numbers to use.
8. Put the unused microplate into an auto-sealing bag to re-seal it. The standard substance and the colorless color former is light sensitive, and thus they cannot be directly exposed to the light.
9. Discard the coloration solution with any color that indicates the degeneration of this solution. The detecting value of the 0 standard solution (0 ppb) of less than 0.5 indicates its degeneration

Work Sheet of Typical Assay-Clenbuterol

Wells	Stds/samples		Mean $A_{450\ nm}$	Absorption Percentage
A1, A2	**Standard A**	0.0 ppb	1.892	100%
B1, B2	**Standard B**	0.1 ppb	1.501	79.33%
C1, C2	**Standard C**	0.3 ppb	1.175	62.10%
D1, D2	**Standard D**	0.9 ppb	0.751	39.69%
E1, E2	**Standard E**	2.7 ppb	0.421	22.25%
F1, F2	**Standard F**	8.1 ppb	0.198	10.47%

NOTE: These data are for demonstration purpose only. A complete standard curve must be run in every assay to determine sample values. Each laboratory should determine their own normal reference values.

In Spain, France, Italy, China, and Portugal had some cases involving individuals who digested meat from animals treated with clenbutarol. The symptoms appeared after about 0.5-3 hours of digestion, they include increase in heart rate, nervousness, headache, muscular tremor, dizziness, nauseas, vomiting, fever and chills. The symptoms went away after a treatment of 2 to 6 days.

Alpha Diagnostic Intl's Clenbuterol ELISA kit is a highly sensitive competitive type assay for the measurement of Clenbuterol in pork liver, serum, urine and feed.

PRINCIPLE OF THE TEST

This test kit is based on the competitive enzyme immunoassay for the detection of Clenbuterol in the pork and liver. The coupling antigen is pre-coated on the micro-well stripes. The Clenbuterol in the sample and the coupling antigens pre-coated on the micro-well stripes compete for the anti-Clenbuterol antibodies. After the addition of the enzyme conjugate, the TMB substrate is added for coloration. The optical density (OD) value of the sample has a negative correlation with the Clenbuterol in the sample. This value is compared to the standard curve and the Clenbuterol residues is subsequently obtained.

MATERIALS AND EQUIPMENT REQUIRED

Equipments: micro plate reader, printer, homogenizer, nitrogen-drying device, vortex, oscillator, centrifuge, measuring pipettes, balance (a sensibility reciprocal of 0.01 g)

Micropipettors: single-channel 20 to 200 µL and 100 to 1000 µL, and multi-channel 250 µL.

Reagents: Acetonitrile (CH_3CN), methanol, NaOH, ethyl acetate, N-hexane, KH_2PO_4 $2H_2O$, HCL (approx 36.5%), tri-deionized water (tri-deionized water should be distilled 3 times for use)

PRECAUTIONS AND SAFETY INSTRUCTIONS

The Clenbuterol Kit is for research use only.

Stop Solution contains 1% sulfuric acid. Follow good laboratory practices, and avoid ingestion or contact of any reagent with skin, eyes or mucous membranes. All reagents may be disposed of down a drain with copious amounts of water.

MSDS for TMB, sulfuric acid, if not already on file, can be requested or obtained from the ADI website.

SAMPLE PRE-TREATMENT

Instructions

The following points must be dealt with before the pre-treatment of any kind of sample:

1. Only the disposable tips can be used for the experiments and the tips must be changed when used for absorbing different reagents.
2. Before the experiment, each experimental equipment must be clean and should be re-cleaned if necessary, in order to avoid the contamination that interferes with the experimental results.

Solution preparation before sample pre-treatment

1. 50 mM HCl: dissolve 4.17 mL HCl (approx 36.5%) in deionized water to 1 L
2. 50 mM (pH=3.0) KH_2PO_4 buffer: dissolve 3.40 g $KH_2PO_4 \cdot 2H_2O$ in deionized water to 500 mL (adjust pH value with H_3PO_4 or NaOH)
3. 500 mM (pH=3.0) KH_2PO_4 buffer: dissolve 34.02 g $KH_2PO_4 \cdot 2H_2O$ in deionized water to 500 mL (adjust pH value with H_3PO_4 or NaOH)
4. 0.1 M HCl: dissolve 0.86 mL HCl (approx 36.5%) in deionized water to 100 mL
5. 1 M HCl: dissolve 8.6 mL HCl (approx 36.5%) in deionized water to 100 mL
6. 0.1 M NaOH: dissolve 0.4 g NaOH in deionized water to 100 mL
7. 1 M NaOH: dissolve 4 g NaOH in deionized water to 100 mL
8. CH_3CN-0.1 M HCl solution: $V_{CH3CN} : V_{HCl}$ =84:16

Samples preparation

a) Urine and serum

Take 20 µL clear urine or serum, directly detect it (If urine and serum are muddy, must filter or centrifuge at 4000 r/min at 15 ℃ for 10 min, then take clear urine and serum). Store at frozen environment if don't use.

b) *First* method of recovery (liver , pork)

1. Weigh 2±0.05 g, add 6 mL CH_3CN-0.1 M HCl solution, vortex for 10 min, centrifuge at 4000 r/min at room temperature (20-25 ℃) for 10 min.
2. Take 3 mL of clear liquid (upper layer), add 2 mL 0.1 M NaOH and 6 mL ethyl acetate, shake for 10 min, centrifuge at 4000 r/min at room temperature (20-25 ℃) for 10 min, take entire supernatant (almost is clear), blow to dry with nitrogen or air at 50 ℃.
3. Add 1 mL tri-deionized water(tri-deionized water should be distilled 3 times for use), redissolve residues properly.
4. Take 20 µL for analysis.

Fold of dilution of sample: 1

C) *Second* method of recovery (liver, pork, tissue)

1. Take 5 ± 0.05 g homogenized sample, add 25 mL 50 mM HCl, shake properly for 1.5 h, vortex it completely.
2. Weigh 6 g homogenized sample (equivalent to 1 g sample), centrifuge at above 4000 r/min at 10-15 ℃ for 15 min.
3. Transfer the supernatant (upper layer) into a new centrifuge tube, add 300 µL 1 M NaOH, mix for 15 min.
4. Add 4 mL 500 mM KH_2PO_4 buffer (pH=3.0), mix properly, store at 4 ℃ for at least above 1.5 h or overnight. Centrifuge at above 4000 r/min at 10-15 ℃ for 15 min, take entire supernatant (must be clear), return it to room temperature (20-24℃), then purify it in RIDA® C 18 column.

Fold of dilution of sample: 1

- purify in RIDA® C 18 column as following method

- Rinse column with 3 mL methanol (100%) at 1 drop/s.
- Rinse column with 2 mL 50 mM (pH=3.0) KH_2PO_4 buffer.
- Put sample into column (meat, liver, tissue must be clear supernatant) at 15 drops/min .
- Rinse column with 2 mL 50 mM (pH=3.0) KH2PO4 buffer.
- Remove fluid residues by positive pressure or vacuum, blow column to dry with nitrogen or air for 2 min.

- Elute sample with methanol(100%) at 5 drops/min.
- Evaporate eluent completely with air or nitrogen at 50-60 ℃.
- Redissolve dried residues with 1 mL tri-deionized water, take 20 µL for analysis.

d) Third method of recovery (for feed)

1. Grind feed sample, weigh 2±0.05 g, add 2 mL 1 M HCl and 16 mL tri-deionized water homogenize it .
2. Vortex for 3 min, then shake with oscillator for 15 min.
3. Centrifuge at above 4000 r/min for 15 min, take entire supernatant (must be clear), add 2 mL 1 M NaOH, mix evenly, adjust pH to 6-8.
4. Centrifuge at above 4000 r/min for 15 min, take entire supernatant (must be clear).
5. Use tri-deionized water to dilute 1:10 (100 µL clear upper liquid + 900 µL tri-deionized water).
6. Take 20 µL for analysis.

Fold of dilution of sample: 100

Sample storage

1. Untreated samples are stored at frozen environment.
2. HCl - acidified homogenates are stable at 2-8 ℃ for up to 3 days
3. Sample treated by KH_2PO_4 buffer (prior to treatment of RIDA® C 18 column) is stable at 2-8 ℃ for 2 days
4. Extracts of methanol purified with RIDA® C 18 column can be stored at frozen condition for 2 months, or at 2-8 ℃ for 2 weeks
5. Prepared sample can be stable at 2-8 ℃ for 1 week.

STORAGE AND STABILITY

Storage: store at 2 to 8 ℃, not frozen.

Expiration date: 12 months; date of production is on box.

TEST PROCEDURE *(ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE FOR AT LEAST 30 min)).*

1. Bring test kit to the room temperature (20-25 ℃) for at least 30 min, note that each reagent must be shaken evenly before use; put the required micro-well strips into plate frames. Resealed the unused microplate, stored at 2-8 ℃, not frozen.
2. Numbering: number the micro-wells according to samples and standard solution; each sample and standard solution should be performed in duplicate; record their positions.
3. Add 20 µL of the sample or the standard solution into separate duplicate wells, then add enzyme conjugate, 50 µL/well; antibody working solution, 80 µL/well, Mix gently by shaking the plate manually, seal the microplate with the cover membrane, incubate at 25 ℃ for 30 min.
4. Pour liquid out of microwell, flap to dry on absorbent paper; add 250 µL/well of tri-deionized water, wash for 4-5 times, marinate for 10 s every time, then take out and flap to dry with absorbent paper.