Antibiotics ELISA kits available from ADI:

DE-100010	Clenbuterol ELISA kit, 96 tests (For Urine, Serum, Feed, Meat, Liver)
DE-100020	Ractopamine ELISA kit, (For Liver, Urine, Feed), 96 tests
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

Instruction Manual No. M-DE-100030

Salbutamol ELISA KIT

Cat. #DE-100030

For Qualitative and Quantitative Determination of Salbutamol in tissue, urine, and feed.

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Salbutamol ELISA KIT Cat. #DE-100030

Kit Components, 96 tests	Cat #
Micro-well coated strip plate (12 strips with 8 removable wells each)	DE-100031
6x standard solution (1 ml each): 0.0 ppb, 0.5 ppb, 1.5 ppb, 4.5 ppb, 13.5 ppb, 40.5 ppb	DE-100032
Enzyme conjugate (12 mL)	DE-100033
Antibody working solution (7 mL)	DE-100034
Substrate A solution (7 mL)	DE-SSA
Substrate B solution (7 mL)	DE-SSB
Stop solution (7 mL)	DE-ST
20x concentrated washing buffer (40 mL)	DE-WB
2x concentrated redissolving solution (50 mL)	DE-SS2
Instruction Manual	M-DE-100030

INTRODUCTION

Salbutamol is also called albuterol is a Beta2-adrenergic receptor agonist which is a class of drugs used on the treatment of asthma and other chronic pulmonary disease. Its chemical formula is $C_{13}H_{21}NO_3$ and its molecular weight is 239.311 g/mol. It is in the market as the name of Ventolin, Aerolin, Ventorlin, Asthalin, Proventil or ProAir depending on the company it was manufactured by. It is usually sold contained in inhalers for asthma suffering patients. Salbutamol relaxes smooth muscle which leads to dilation of bronchial passages, vasodilation in muscle and liver. It is also used in obstetrics to relax the uterine muscle to delay premature labor. Beta2 agonists relax bronchial smooth muscle, decrease mast cell degranulation and histamine release, inhibit microvascular leakage into the airways and increase mucociliary clearance. Side effects of this drug include tremor, nervousness, headache, muscle cramps, dry mouth, and palpitation. Other side effects might include tachycardia, arrhythmias, flushing, myocardial ischaemia, and disturbances of sleep and behavior.

In April of 2005 the United States Food & Drug Administration announced that as December 31, 2008, all inhalers including salbutamol and chlorofluorocarbons (CFCs) would be banned in the United States. CFC is a group of haloalkanes which have shown to have been destroying the ozone layer.

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

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 Salbultamol concentration.

Qualitative determination

The concentration range (ng/mL) can be 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 0.5 ppb, 1.175 for 1.5 ppb, 0.751 for 4.5 ppb, 0.421 for 13.5 ppb ,0.198 for 40.5 ppb, accordingly the concentration range of the sample Ⅰ is 13.5 to 40.5 ppb, and that of the sample Ⅱ is 1.5 to 4.5 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,

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

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

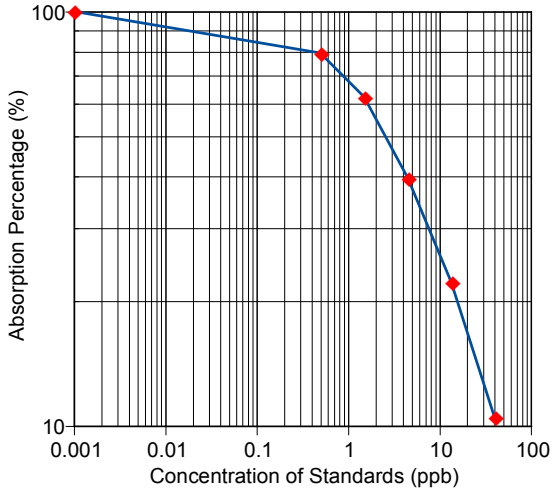
Draw the standard curve with the absorption percentages of the standard solution and the semilogarithm values of the Sabutamol standard solution (ng/mL) as Y- and X-axis, respectively. Read the corresponding concentration of the sample from the standard curve by incorporating its absorption percentage into the standard curve. The resulting value is subsequently multiplied by the corresponding dilution fold, finally obtaining the Sabutamol 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).

Technical specifications
Sensitivity: 0.5 ppb
Detection limit
Tissue..... 0.5 ppb
Urine, serum.....0.5 ppb
Feed.....50 ppb
Recovery rate
Urine, serum..... 90±10%
Tissue.....80±10%
Feed.....80±15%
Cross-reaction rate
Salbutamal.....00%
Terbutalin.....< 1%
Clenbuterol.....< 13%
Ractopamine.....< 1%

Work Sheet of Typical Assay-Salbutamol

Wells	Stds/samples		Mean $A_{450\ nm}$	Absorption Percentage
A1, A2	**Standard A**	0.0 ppb	1.892	100%
B1, B2	**Standard B**	0.5 ppb	1.501	79.33%
C1, C2	**Standard C**	1.5 ppb	1.175	62.10%
D1, D2	**Standard D**	4.5 ppb	0.751	39.69%
E1, E2	**Standard E**	13.5 ppb	0.421	22.25%
F1, F2	**Standard F**	40.5 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.



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

PRINCIPLE OF THE TEST

The test kit is based on the competitive enzyme immunoassay for the detection of Salbutamal in the sample. The coupling antigen is pre-coated on the micro-well stripes. The Salbutamal in the sample and the coupling antigens pre-coated on the micro-well stripes compete for the anti-Salbutamal 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 Salbutamal in it. This value is compared to the standard curve and the Salbutamal residues is subsequently obtained.

MATERIALS AND EQUIPMENT REQUIRED

Equipments: microplate reader, printer, homogenizer, nitrogen-drying device, vortex, oscillator, centrifuge, measuring pipettes, balance (a sensibility reciprocal of 0.01 g)
Micropipettes: single-channel 20 to 200 µL and 100 to 1000 µL, and multi-channel 250 µL.
Reagents: Acetonitrile (CH_3CN), NaOH, ethyl acetate, N-hexane, HCl (approx 36.5%), Isopropanol

PRECAUTIONS AND SAFETY INSTRUCTIONS

The Salbutamol 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. 1 M HCl: dissolve 8.6 mL HCl (approx 36.5%) in deionized water to 100 mL.
2. 1 M NaOH: dissolve 4 g NaOH in deionized water to 100 mL.
3. The 2× concentrated redissolving solution is mixed with deionized water at 1:1 (1 mL concentrated redissolving solution + 1 mL deionized water, for redissolving) and 1:3 (for extracting sample).

Samples preparation

a) Urine and serum

Take 50 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 if don't use. Some interference in urine, we recommend 1 ppb as cut off value of positive sample.

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

1. Weigh 2 ± 0.05 g sample, add 1 mL 0.5× concentrated redissolving solution (diluted at 1:3), mix properly, then add 1 mL isopropanol, vortex for 10 min, centrifuge at 4000 r/min at room temperature (20-25 ℃) for 10 min.
2. Take 3 mL supernatant, add 50 µL 1 M NaOH, mix properly, then add 7 mL Ethyl acetate solution, shake for 10 min, centrifuge at 4000 r/min at room temperature (20-25 ℃) for 10 min, take all supernatant, blow to dry with nitrogen or air at 50 ℃.
3. Dissolve residues in 1 mL 1×diluted redissolving solution (diluted at 1:1), shake properly, centrifuge at room temperature (20-25 ℃) for 10 min.
4. Take 50 µL for analysis.

Fold of dilution of sample: 1

c) *Second* method of recovery (feed)

1. Take 2 ± 0.05 g grinded sample, add 2 mL 1 M HCl, 16 mL deionized water, homogenize.
2. Vortex for 3 min, shake for 15 min with oscillator .
3. Centrifuge at 4000 r/min for 15 min, take the supernatant (upper layer) , add 1 mL 1 M NaOH, adjust PH value to 6-8.
4. Centrifuge at 4000 r/min for 15 min(If it's not clear, should be centrifuged at higher speed).
5. Dilute supernatant with diluted redissolving solution at 1:9 (100 µL supernatant + 900 µL diluted redissolving solution).
6. Take 50 µL for further analysis.

Fold of dilution of sample: *100*

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).*

Instructions

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. Solution preparation: dilute 40 mL of the concentrated washing buffer (20 × concentrated) with the distilled or deionized water to 800 mL (or just to the required volume) for use.

3. Numbering: number the micro-wells according to samples and standard solution; each sample and standard solution should be performed in duplicate; record their positions.
4. Add 50 µL of the sample or standard solution to separate duplicate wells, and add antibody working solution, 50 µL/well, seal the microplate with the cover membrane, and incubate at 37 ℃ for 30 min.
5. Pour the liquid out of microwell, wash the microplate with the washing buffer at 250 µL/well for four to five times. Each time soak the well with the washing buffer for 15 sec, flap to dry with absorbent paper (if there are the bubbles after flapping, cut them with the clean tips).
6. Add 100 µL enzyme conjugate into each well, seal the microplate with the cover membrane, and incubate at 37 ℃ for 30 min, continue as described in 5.
7. 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 37 ℃ for 15 min at dark for coloration.
8. Determination: add 50 µL 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.
2. Return all reagents to 2-8 ℃ immediately after use.
3. 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.
4. 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.
5. 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.
6. 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.
7. Mix evenly, otherwise there will be the undesirable reproducibility.
8. The stop solution is the 2 M sulfuric acid solution, avoid contacting with the skin.
9. 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.
10. 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.
11. 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.