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-100130	Enrofloxacin 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

Life Technologies™
Your Molecular & Cell Technology Partner

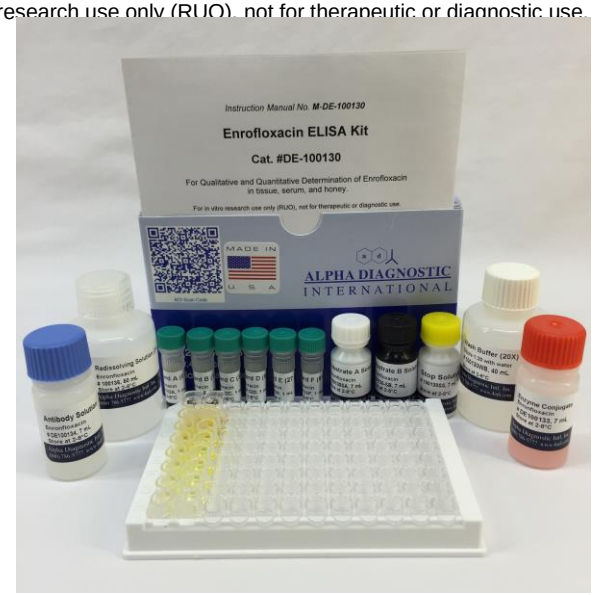
Instruction Manual No. ***M-DE-100130***

Enrofloxacin ELISA Kit

Cat. #DE-100130

For Qualitative and Quantitative Determination of Enrofloxacin in tissue, serum, and honey.

For in vitro research use only (RUO), not for therapeutic or diagnostic use.



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Enrofloxacin ELISA KIT Cat. #DE-100130

Kit Components, 96 tests	Cat #
Micro-well **coated strip plate** (12 strips with 8 removable wells each) # DE100131	1 plate
6x **standards** (1 ml each): #DE100132A-F 0.0 ppb, 1 ppb, 3 ppb, 9 ppb, 27 ppb, 81 ppb	6 vials
Enzyme conjugate (7 mL) # DE100133; red cap	1 bottle
Antibody solution (7 mL) # 100134; blue cap	1 bottle
redissolving solution (2X), 50 ml #100135; transparent cap	1 bottle
Washing buffer (20X), 40 ml, 100130WB; white cap	1 bottle
Substrate A solution (7 mL) 100130SA; white cap	1 bottle
Substrate B solution (7 mL) 100130SB; black cap	1 bottle
Stop solution (7 mL) 100130SS; yellow cap	1 bottle
Instruction Manual	M-DE100130

Intended use

Alpha Diagnostic Intl's Enrofloxacin ELISA kit is a highly sensitive competitive type ELISA for the measurement of Enrofloxacin in tissue, serum and honey. For in vitro research use only (RUO), not for therapeutic or diagnostic use.

Introduction

Enrofloxacin is a synthetic antibiotic derived from the class of fluoroquinolone carboxylic acid. Fluoroquinolone is a quinolone with a fluorine atom attached to the central ring system. Quinolone is derived from chloroquine and is used as a chemotherapeutic agent to treat serious, complicated, life threatening bacterial infections. Enrofloxacin is an antibiotic sold by Bayer Corporation as the name of Baytril. It is currently FDA approved to treat pets and domestic animals in the United States. Enrofloxacin is widely effective within gram-positive and gram-negative organisms. Enrofloxacin mechanism works by interfering with the bacteria DNA metabolism and inhibiting two enzymes called topoisomerase II in gram-negative and topoisomerase IV in gram-positive. Topoisomerases are enzymes that uncoil and coil DNA to help control and synthesize the production of protein. It can be used to treat Pseudomonas aeruginosa, Klebsiella, E. coli, Enterobacter, Campylobacter, Shigella, Salmonella, Aeromonas, Haemophillus, Proteus, Yersinia, Serratia, Vibrio, Brucella, Chlamydia, Staphylocci, Mycoplasma, and Mycobacterium. It does not work well against anaerobic bacteria.

Enrofloxacin is approved to be used in dogs and cats to treat infections such as, skin infections, urinary tract infections, respiratory infections and wound infections caused by bacteria. If given in high doses it can cause articular cartilage abnormalities in dogs. Side effects include vomiting, diarrhea, and elevated liver enzymes.

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 Enrofloxacin concentration.

Qualitative determination

The concentration range (ng/mL) obtained from the comparison the average OD value of the sample with that of the standard solution. Assuming that the OD value of the sample Ⅰ is 0.238, and that of the sample Ⅱ is 0.946, the OD value of standard solutions is: 1.845 for 0 ppb, 1.542 for 1 ppb, 1.130 for 3 ppb, 0.635 for 9 ppb, 0.326 for 27 ppb ,0.156 for 81 ppb, accordingly the concentration range of the sample Ⅰ is 27 to 81 ppb, and that of the sample Ⅱ is 3 to 9 ppb. (multiplied by the corresponding dilution fold).

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 Enrofloxacin 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 Enrofloxacin 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: 1 ppb

Detection limit
Tissue.....1 ppb
Serum.....2 ppb
Honey.....2 ppb

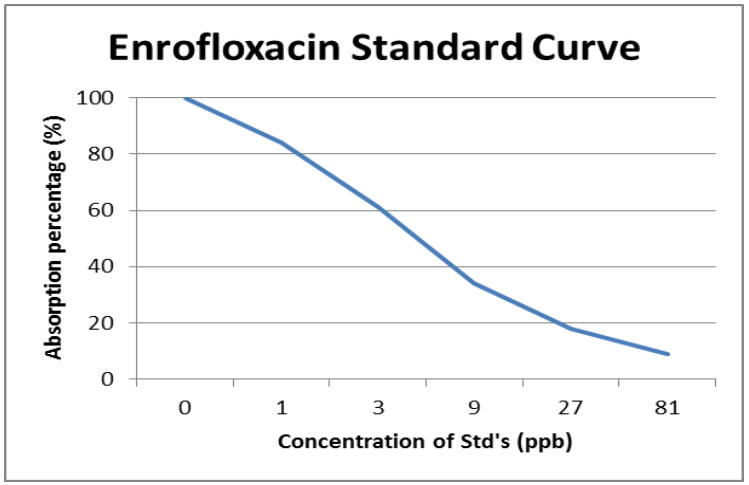
Recovery rate
Tissue.....80±15%
Serum.....80±15%
Honey.....75±15%

Cross-reactivity
Enrofloxacin.....100%

Work Sheet of Typical Assay-Enrofloxacin

Wells	Stds/samples		Mean A_{450} nm	Absorption Percentage
A1, A2	**Standard A**	0.0 ppb	1.845	100%
B1, B2	**Standard B**	1 ppb	1.542	83.57%
C1, C2	**Standard C**	3 ppb	1.130	61.24%
D1, D2	**Standard D**	9 ppb	0.635	34.41%
E1, E2	**Standard E**	27 ppb	0.326	17.66%
F1, F2	**Standard F**	81 ppb	0.156	8.45%

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

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

MATERIALS AND EQUIPMENT REQUIRED

Equipments: microplate reader, printer, vortex, centrifuge, homogenizer, measuring pipettes and balance (a sensibility reciprocal of 0.01 g)
Micropipettors: single-channel 20 to 200 µL and 200 to 1000 µL, and multi-channel 250 µL.
Reagents: HCl, Methylene chloride, Acetonitrile (CH_3CN), N-hexane, $Na_2HPO_4{\cdot}12H_2O$, $NaH_2PO_4{\cdot}2H_2O$, Heparin sodium

PRECAUTIONS AND SAFETY INSTRUCTIONS

The Enrofloxacin Kit is for research use only.

Applicable **MSDS**, if not already on file, for the following reagents can be obtained from ADI web site. TMB (substrate), H2SO4 (stop solution), and Prolcin-300 (0.1% v/v in standards, sample diluent and HRP-conjugates).

SAMPLE PRE-TREATMENT

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

1. This test kit can detect tissue sample: animal tissue, poultry, aquatic. Eg: Chicken, duck, bovine, rabbit, fish, shrimp etc.
2. Only the disposable tips can be used for the experiments and the tips must be changed when used for absorbing different reagents.
3. 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 required but not provided

1) 0.1 M HCl: 860µl HCl (36%) + deionized water 100 mL.
2) Acetonitrile (CH_3CN) -Methylene chloride mixing solution $V_{acetonitrile}$-$V_{methylene\ chloride}$ = **1 : 4**
3) pH7.2 0.02M PB buffer: dissolve 5.16 g $Na_2HPO_4{\cdot}12H_2O$ + 0.87 g $NaH_2PO_4{\cdot}2H_2O$ in the deionized water to 1 L.
4) Acetonitrile (CH_3CN)- Methylene chloride- 0.1 M HCl mixing solution 100ml Acetonitrile (CH_3CN) -Methylene chloride mixing solution ($V_{acetonitrile}$-$V_{methylene\ chloride}$ **= 4 : 1)**, add 5ml 0.1 M HCl solution.

Samples preparation

a) Tissue (Chicken/liver, pork/liver, fish, shrimp etc)

1) Weigh 2.0 ± 0.05 g of the homogenized tissue sample into 50 ml centrifuge tube

2) Add 8 ml of the Acetonitrile (CH3CN) -Methylene chloride mixing solution, shake for 5 min, centrifuge at above 4000 r/min at 15 ℃ for 10 min

3) Take 4 ml the clear organic phase (upper layer) into a dry tube, blow to dry with nitrogen or air completely by rotary evaporation at 56 ℃.

4) Dissolve the dry residues in 1 mL of the diluted redissolving solution, add 1 mL N-hexane, mix for 30 seconds; centrifuge at above 4000 r/min at 15 ℃ for 5 min.

5) Remove the upper layer, take 50μl lower layer solution for further analysis.

Fold of dilution of the sample: 1

b) Serum

1) Use centrifuge tube with heparin sodium (20-30 unit/ml blood) to collect chicken blood sample (Suggestion: blood collection syringes are recommended rinsing with heparin).Place the blood sample in the room temperature for 1 hour. After obtain plasma, centrifuge at above 4000r/min at 15 ℃ for 10 min, take out 1 ml plasma.
2) Add CH3CN (without water) 4 ml , mix up-and-down thoroughly for 5 min, centrifuge at above 4000r/min at 15 ℃ for 10 min.
3) Move the clear supernatant (upper layer) to another centrifuge tube, add 2ml 0.02M PB buffer, mix evenly.
4) Add 5 ml Methylene chloride, mix evenly for 5 min, centrifuge at above 4000r/min at
5) 15 ℃ for 10 min, remove the upper layer, take the lower organic phase to dry bottle(clear without impurities), blow to dry with nitrogen or air completely by rotary evaporation at 50 ℃
6) Dissolve the dry residues in 1 mL of the diluted redissolving solution, add 1 mL N-hexane, mix for 30 seconds; centrifuge at above 4000 r/min at 15℃ for 5 min.
7) Absorb out lightly the upper and middle layer white impurities, take lower phase 100μl, add 100μl diluted redissolving solution, mix for 30s.
8) Take 50μl solution for further analysis.

Fold of dilution of the sample: 2

d) Honey, Royal jelly

1) Weigh 2.0±0.05g honey sample into 50ml centrifuge tube, add 8 ml Acetonitrile (CH3CN)- Methylene chloride- 0.1 M HCl mixing solution, shake fully for 3min, centrifuge at above 4000 r/min at 15 ℃for 10 min.
2) Take 2ml the supernatant (upper layer), blow to dry with nitrogen or air at 56 ℃.
3) Add 1 mL the diluted redissolving solution, shake fully for 1min.
4) Take 50 μL for further analysis
5) Fold of dilution of the sample: 2

STORAGE AND STABILITY

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

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

Reagent Preparation

Wash buffer: Dilute 1:20 with water (40 mL 20X mixed with 760 ml water. Or Prepare as needed. Store 1X wash buffer at room temp for the duration of the use and store at 4oC until the kit expiration.

Redissolving solution (2X): Dilute 1:1 with water (50 mL 2X mixed with 50 ml water. Or Prepare as needed. Store 1X buffer at room temp for the duration of the use and store at 4oC until the kit expiration.

Assay Procedure

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.

1. Add **50 μL** of **the standard and sample in** duplicate wells.

2. Add **50 μL** of **the antibody solution** to each well, mix gently manually for 5-10 seconds. Cover the microplate and incubate at **25°C for 30 min**.

3. Aspirate the plates and tap dry on absorbent paper. Wash the plate 5 times with 300 ul wash buffer (1X).

4. Add **50 μL enzyme conjugate** to each well. Mix gently manually for 5-10 second, cover the plate and incubate at **25 °C for 30 min**. Aspirate the plates and tap dry on absorbent paper**. Wash 5 times** same as in step 3.

5. Add **50 μL** of the **substrate A** solution followed by **50 μL of the B solution** into each well. (alternatively it is possible to mix Soln A and B in 1:1 ratio in a clean tube, mix, and add 100 ul of the mix in a single step). Mix gently manually for 5-10 seconds. Cover the plate and incubate at **25 °C for 15-20 min** in the dark (blue color develops in standards and samples).

6. Add **50 μL** of the **stop solution** into each well. Mix gently manually for 5-10 seconds (blue color turns yellow). Read the plate at 450 nm to determine the OD value of every well. (Recommend to read the OD value at the dual-wavelength 450/630nm) within 5-10 min.

NOTES:

1. Return all reagents to 2-8 °C 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. 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.
5. Mix evenly, otherwise there will be the undesirable reproducibility.
6. The stop solution contains 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 standard solution 1（0 ppb）of less than 0.5 indicates its degeneration.
10. Substrate time is about 20 min, if the color is light , prolong the time of coloration but don't exceed 30 min.
11. The optimum reaction temperature is 25 °C, and too high or low temperatures will result in the changes in the detecting sensitivity and OD values.