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

See Details at the web site or Contact ADI

Life Technologies™
Your Molecular & Cell Technology Partner

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Instruction Manual No. M-DE-100050

Florfenicol ELISA KIT

Cat. #DE-100050.

For Qualitative and Quantitative Determination of Florfenicol in animal tissue, aquatic and honey.

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Florfenicol ELISA KIT Cat. # DE-100050

Kit Components, 96 tests	Cat #
Micro-well coated strip plate (12 strips with 8 removable wells each)	DE-100051
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-100052
Enzyme conjugate (12 mL)	DE-100053
Antibody working solution (7 mL)	DE-100054
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
20× concentrated redissolving solution (50 mL)	DE-SS20
Instruction Manual	M-DE-100050

INTRODUCTION

Florfenicol is a synthetic drug made from the antibiotic thiamphenicol with a fluoride added to it. It is in the market as the name of Nuflor by Schering-Plough Animal Health. Florfenicol has a molecular weight of 358.21 g/mol and its chemical formula is $C_{12}H_{14}Cl_2FNO_4S$. It works as bacteriostatic and acts by binding to the 50S ribosomal subunit and inhibiting bacterial protein synthesis against many gram-negative and gram-positive bacteria. In the United States, florfenicol is used to treat bovine respiratory disease related to Mannheimia (Pasteuralla) haemolytica, Pasteurella multocida, and Haemophilus somnus. It is also used to treat interdigital phlegmon related to Fusobacterium necrophorum and bacteroides melaninogenicus. Interdigital phlegmon is also called foot rot a disease that is caused by the inflammation of the sensitive tissues of the feet and severe lameness. Interdigital phlegmon is infected by the bacteria Fusobacterium necrophorum and also Bacteroides melaninogenicus have shown similar symptoms. The disease seems to be contagious and happens more frequently during the rain in summer and fall, when the weather is very humid and the ground is too wet. When the ground is too wet cuts on the foot become very common and bacteria enter through those cuts into the skin on the lower part of the foot.

The targets for the disease are cattle of all ages but more common in adults. It is more common among Brahman or Zebu-type cattle and British breeds seems to be more resistant. Visible symptoms of the disease on the cow include severe foot lameness, fiver between 103 to 104 F, and decrease of milk production.

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 florfenicol 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 0.250, and that of the sample Ⅱ is 0.720, the OD value of standard solutions is: 1.610 for 0 ppb, 1.380 for 0.5 ppb, 1.100 for 1.5 ppb, 0.620 for 4.5 ppb, 0.289 for 13.5 ppb, 0.108 for 40.5 ppb, accordingly the concentration range of the sample Ⅰ is 13.5 to 40.5, 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 Florfenicol 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 Florfenicol 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
Honey.....1.5 ppb
Meat, liver, shrimp, fish..... 0.5 ppb

Recovery rate
Honey..... 70 ±10%
Meat, liver, shrimp, fish.....80 ±10%

Cross-reaction rate
Florfenicol..... 100%
Florfenicol Amine..... 11%
Thiamphenicol..... < 0.1%
Chloramphenicol.....< 0.1%

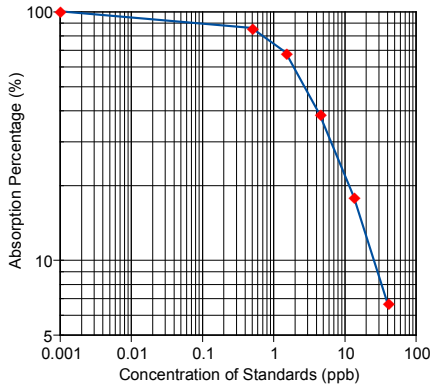
NOTES:

1. 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.
2. Dryness of the microplate in the washing process will be accompanied by the situations including the non-linear standard curves and the undesirable reproducibility.
3. Mix reagent and wash the microplate thoroughly, otherwise there will be the undesirable reproducibility.
4. The stop solution is the 2 M sulfuric acid solution, avoid contacting with the skin.
5. 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 exposed to the light.
6. 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.
7. Discard the coloration solution with any color that indicates the degeneration of this solution. The detecting value of the 0 standard solution of less than 0.5 indicates its degeneration.
8. The optimum reaction temperature is 37 ℃, and too high or too low temperatures will result in the changes in the detecting sensitivity and OD values.

Work Sheet of Typical Assay-Florfenicol

Wells	Stds/samples		Mean $A_{450\ nm}$	Absorption Percentage
A1, A2	**Standard A**	0.0 ppb	1.610	100%
B1, B2	**Standard B**	0.5 ppb	1.380	85.71%
C1, C2	**Standard C**	1.5 ppb	1.100	68.32%
D1, D2	**Standard D**	4.5 ppb	0.620	38.51%
E1, E2	**Standard E**	13.5 ppb	0.289	17.95%
F1, F2	**Standard F**	40.5 ppb	0.108	6.71%

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)

The concerns that bring the use of florfenicol treatment are the residues can lead to aplastic anemia. Aplastic anemia is a condition where bone marrow does not produce enough cells. People who suffer from aplastic anemia have low counts of red blood cells, white blood cells, and platelets. This condition will make you feeling very fatigue, gets easily infected and uncontrolled bleeding.

Alpha Diagnostic Intl's Florfenicol ELISA kit is a highly sensitive competitive type assay for the measurement of Florfenicol in animal tissue, aquatic and honey.

PRINCIPLE OF THE TEST

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

MATERIALS AND EQUIPMENT REQUIRED

Equipments: microplate reader, printer, mixer or stomacher, nitrogen-drying device, oscillator, centrifuge, measuring pipettes, and 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: Ethyl acetate, N-hexane, deionized water.

PRECAUTIONS AND SAFETY INSTRUCTIONS

The Florfenicol 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

Solution I： the 2× concentrated redissolving solution is mixed with deionized water at 1:1 (1 mL

Samples preparation

a) Shrimp, fish and meat

1. Homogenize the sample, use stomacher or mixer.
2. Weight 3.0 ± 0.05 g of the homogenized sample, add 6 mL of ethyl acetate, shake properly for 10 min, centrifuge at above 4000 r/min at room temperature for 10 min.
3. Take 2 mL of the supernatant (upper layer, equivalent to 1 g sample), blow to dry with nitrogen at 50-60 ℃.
4. Dissolve the dry residues in 1 mL of the diluted redissolving solution, add 1 mL N-hexane, shake vigorously for 1 min; centrifuge at above 4000 r/min at room temperature for 15 min.
5. Take 50 µL of the lower, aqueous phase for analysis.

Fold of dilution of the sample: one

b) Honey

1. Put 2±0.05 g into centrifugal tube, add 4 mL deionized water.
2. Add 4 mL ethyl acetate, shake upside and down for 10 min.
3. Centrifuge at above 4000 r/min at room temperature for 10 min.
4. Transfer 1 mL of ethyl acetate upper layer (equivalent to 0.5 g sample) to a new vessel, reduce to dryness with nitrogen at 50-60 ℃.
5. Add 0.5 mL of the diluted redissolving solution.
6. Take 50 µL for analysis.

Fold of dilution of the sample: one

Detection limit: 0.5 ppb **Quantitative limit :**1.5 ppb

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 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.

Operation procedure

1. Take out all the necessary reagents from 4 ℃ environment, bring them to the room temperature (20-25 ℃) for at least 30 min, note that each liquid reagent must be shaken evenly before use.
2. Take the required micro-well strips and plate frames. Re-sealed the unused microplate, store at 2-8℃, not frozen.
3. Solution preparation: dissolve the 20×concentrated washing buffer with the distilled or deionized water to 800 mL (or just to the required volume) for use.
4. Numbering: number the micro-wells according to samples and standard solution; each testing sample and standard solution should be performed in duplicate; record their positions.
5. Add 50 µL of the sample or standard solution to separate duplicate wells, add 50 µL of the antibody working solution into each well. Vortex evenly, seal the microplate with the cover membrane, and incubate at 37 ℃ for 30 min.
6. Wash the microplate with the washing buffer at 250 µL/well for 4-5 times. Each time soak the well with the washing buffer for 10s and then flap to dry (if there are the bubbles after flapping, cut them with the clean tips).
7. Add 100 µL of the enzyme conjugate, seal the microplate with the cover membrane, incubate at 37 ℃ for 30 min, pour the liquid out of the wells, continue as described in step 6.
8. Coloration: add 50 µL of the substrate solution and then 50 µL of the B solution into each well. Mix gently by shaking the plate manually, and incubate at 37 ℃ for 15 min at dark for coloration.
9. 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 (we recommend to read the OD value at the dual-wavelength 450/630 nm within 5 min).