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

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Instruction Manual No. M-DE-100230

Olaquindox ELISA KIT

Cat. #DE-100230.

For Qualitative and Quantitative Determination of Olaquindox in tissue and feed

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Olaquindox ELISA KIT Cat. #DE-100230

Kit Components, 96 tests	Cat #
Micro-well coated strip plate (12 strips with 8 removable wells each)	DE-100231
6x standard solution (1 ml each): 0 ppb, 1 ppb, 3 ppb, 9 ppb, 27 ppb, 81 ppb	DE-100232
Enzyme conjugate (12 mL)	DE-100233
Antibody working solution (7 mL)	DE-100234
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-100230

INTRODUCTION

Olaquindox is a quinoxaline 1,4-dioxide. Its chemical formula is $C_{12}H_{13}N_3O_4$ and its molecular weight is 263.249 g/mol. Quinoxaline is also called benzopyrazine a heterocyclic compound containing a benzene ring and a pyrazine ring. Quinoxalines are used as dyes, and as antibiotics such as echinomycin, neomycin and actinoleutin.

Olaquindox is usually added in pig farming used as a growth promoter. It will work against coli form bacteria by inhibiting DNA synthesis. Coliform bacteria are bacterial indicator of quality control in foods and water. They are rod-shaped gram-negative non-spore forming organisms. Coliforms are mostly found in feces of warm-blooded animals. Coliforms can also indicate the presence of other fecal pathogens such as bacteria, viruses, protozoa and parasites.

Olaquindox can cause adrenal damage at growth promoting levels. Depending on the animal metabolites of olquindox vary. One of the metabolites 3-methylquinoxaline-2-carboxilic acid is known to be mutagenic. The drug is rapidly absorbed by the gut and excreted through urine. Olaquindox is genotoxic and can be harmful to people who handle it or to the animal that digests.

Alpha Diagnostic Intl's Olaquindox ELISA kit is a highly sensitive competitive type assay for the measurement of Olaquindox in tissue 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 olaquindox concentration.

Qualitative determination

The concentration range (ng/mL) can be 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 0.313, and that of the sample Ⅱ is 1.032 , while those of the standard solutions are as the followings: 1.892 for 0 ppb, 1.501 for 1 ppb, 1.175 for 3 ppb, 0.751 for 9 ppb, 0.421 for 27 ppb and 0.198 for 81 ppb, accordingly the concentration range of the sample Ⅰ is 27 to 81ppb, and that of the sample Ⅱ is 3 to 9 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

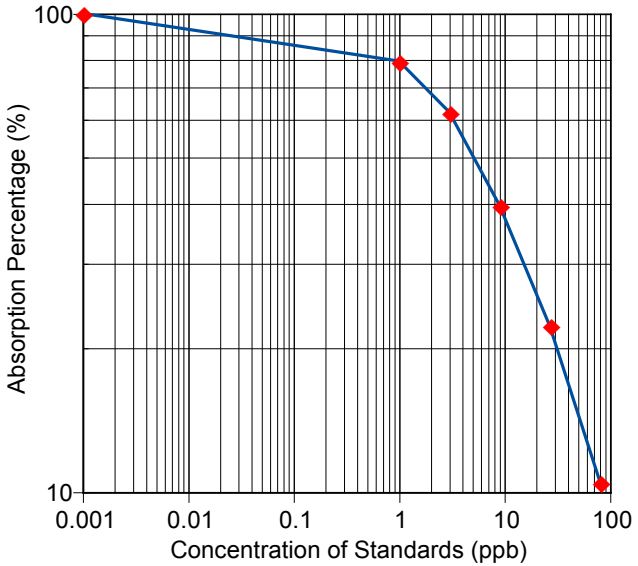
Draw the standard curve with the absorption percentages of the standard solution and the semilogarithm values of the olaquindox standard solution (ng/mL) as Y- and X-axis, respectively. Read the corresponding concentration of the sample from the standard curve by incorporating its absorption percentage into the standard curve. The resulting value is subsequently multiplied by the corresponding dilution fold, finally obtaining the olaquindox 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
Feed…….....100 ppb
Recovery rate
Tissue.....80±10%
Feed…….....80±15%
Cross-reaction rate
Olaquindox.....100%
Carbodox.....<7%

Work Sheet of Typical Assay-Olaquindox

Wells	Stds/samples		Mean $A_{450\ nm}$	Absorption Percentage
A1, A2	**Standard A**	0 ppb	1.892	100%
B1, B2	**Standard B**	1 ppb	1.501	79.33%
C1, C2	**Standard C**	3 ppb	1.175	62.10%
D1, D2	**Standard D**	9 ppb	0.751	39.69%
E1, E2	**Standard E**	27 ppb	0.421	22.25%
F1, F2	**Standard F**	81 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

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

MATERIALS AND EQUIPMENT REQUIRED

Equipments: microplate reader (450 nm / 630 nm), oscillator, vortex, centrifuge, homogenizer, 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: NaOH, Ethyl acetate, HCl (approx 36.5%), CH_3CN

PRECAUTIONS AND SAFETY INSTRUCTIONS

The Olaquindox 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. 0.1 M HCl: dissolve 0.86 mL HCl (approx 36.5%) in water to 100 mL
2. 0.1 M NaOH: dissolve 0.4 g NaOH in water to 100 mL(for feed sample).
3. Acetonitrile-HCl solution: $V_{acetonitrile}$:V_{H2O} = 84:16 (add 84 mL acetonitrile and 16 mL 0.1 M HCl, mix evenly)(For tissue sample).

Samples preparation

a) Liver and meat sample

1. Take 2± 0.05 g of the homogenized sample into tube, add 6 mL of acetonitrile-HCl solution, shake properly with oscillator for 10 min.
2. Centrifuge at above 4000 r/min at room temperature (20 - 25 ℃) for 10 min.
3. Take 3 mL supernatant, add 2 mL of 0.1 M NaOH and 6 mL ethyl acetate, shake for 10min.
4. Centrifuge at above 4000 r/min at room temperature (20-25 ℃) for 10 min, take all the supernate and then blow to dry with nitrogen at 50℃.
5. Dissolve the dry residues in 1 mL of the diluted redissolving solution
6. Take 50 µL for analysis.

Fold of dilution of the sample: 1

b) Feed

1. Weigh 1± 0.05 g homogenized sample into centrifuge tube, add 10 mL deionized water, vortex to be dissolved, then incubate for 30 min at 70 ℃.
2. Centrifuge at above 4000 r/min for 15 min, take the supernate (If the supernate is still not clear, should improve the Rotationl Speed or have a filter).
3. Dilute the supernate (add 100 µL supernate to 0.9 mL redissolving solution)
4. Dissolve the dry residue in 1 mL of the diluted redissolving solution
5. Take 50 µL for analysis.

Fold of dilution of the 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. Take out all the necessary reagents from the kit and place at the room temperature (20 to 25 ℃) for at least 30 min. Note that each liquid reagent must be shaken to mix evenly before use
2. Take the required micro-well strips and plate frames. Re-sealed the unused microplate, stored at 2-8 ℃, not frozen.
3. Solution preparation: dilute 40 mL of 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 sample and standard solution should be performed in duplicate; record their positions.
5. Add 50µL of the sample and standard solution to separate duplicate wells, and add 50 µL of the antibody solution into each well. Mix gently by shaking the plate manually, seal the microplate with the cover membrane, and incubate at 37 ℃ for 30 min.
6. Pour liquid out of microwell, add 250 µL/well of washing buffer for 10 sec, repeat four to five times, then flap to dry (if there are the bubbles after flapping, cut them with the clean tips).
7. Enzyme conjugate: Add enzyme conjugate, 100 µL each well. seal the microplate with the cover membrane, and incubate at 37 ℃ for 30 min. Pour liquid out of microwell, add 250 µL/well of washing buffer for 10 sec, repeat five times, then flap to dry (if there are the bubbles after flapping, cut them with the clean tips).
8. Coloration: add 50 µL of the substrate A 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 (Recommend to read the OD value at the dual-wavelength 450/630 nm within 5 min).

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 every reagent and reaction mixture evenly 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 directly 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
7. reagents from the kits of different lot numbers to use
8. 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
9. 20 min for coloration after addition of substrate A and B, if the coloration is light, prolong time and don't exceed 30 min.
10. 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.
11.