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

Instruction Manual No. M-DE-100070

Aminohydantoin (AHD) ELISA KIT

Cat. # DE-100070.

For Qualitative and Quantitative
Determination of Aminohydantoin
in fish, shrimp, honey, and chicken liver.

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Aminohydantoin (AHD) ELISA KIT Cat. # DE-100070

Kit Components, 96 tests	Cat #
Micro-well coated strip plate (12 strips with 8 removable wells each)	DE-100071
6x standard solution (1ml each): 0.0 ppb, 0.05 ppb, 0.15 ppb, 0.45 ppb, 0.45 ppb, 1.35 ppb and 4.05 ppb	DE-100072
Enzyme conjugate (12ml)	DE-100073
Antibody working solution (7ml)	DE-100074
Substrate A solution (7ml)	DE-SSA
Substrate B solution (7ml)	DE-SSB
Stop solution (7ml)	DE-ST
20x Concentrated Washing buffer (40ml)	DE-WB
20× concentrated redissolving solution (50 mL)	DE-SS20
2-Nitrobenzaldehyde (10ml)	DE-100075
Instruction Manual	M-DE-100070

INTRODUCTION

Aminohydantoin (AHD) is the metabolite residue of nitrofurantoin. There are four main types of nitrofuran, they are: furazolidone, furaltadone, nitrofurazone and nitrofurantoin.

Nitrofurantoin chemical formula is $C_8H_6N_4O_5$ and molecular weight is 238.16g. It is an antibiotic widely used in human medicine to treat cysts and other urinary tract infections. It is also used in veterinary practice as an antibiotic and growth promoter. It can kill various gram-positive and gram-negative bacteria, which are mainly found in animal and aquaculture production. For that reason in some countries it is widely used mixed in animals' water and food. Nitrofurantoin residue, AHD, can be passed through the food chain to human. AHD can cause cancer, teratogenesis, and other side effects. The mechanism of the drug works by interfering with various chemical reactions in the bacteria and damaging its DNA. Since its high toxicity on February of 2002 FDA banned the use of any nitrofuran drug in food-producing animals.

AHD is very stable in tissue so it will be a good source to be detected on fish, shrimp, honey and chicken liver. AHD can be detected by LC-UV, LC-MS, or LC-MS/MS methods, however AHD ELISA is a more sensitive and low cost tool to detect the use of aminohydantoin.

Alpha Diagnostic Intl's Aminohydantoin (AHD) ELISA kit is a highly sensitive competitive type assay for the measurement of Aminohydantoin in fish, shrimp, honey, and chicken liver.

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 content of AHD.

QUALITATIVE RESULTS

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 is 0.238, and that of the sample is 1.130ppb, the OD value of standard solutions is: 1.821 for 0 ppb, 1.484 for 0.05 ppb, 1.163 for 0.15 ppb, 0.657 for 0.45 ppb, 0.290 for 1.35 ppb ,0.116 for 4.05 ppb, accordingly the concentration range of the sample is 1.35 to 4.05 ppb, and that of the sample is 0.15 to 0.45 ppb.

QUANTITAVE RESULTS

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 (double wells) OD value of the sample or the standard solution
B0—the average OD value of the 0ng/mL standard solution

Draw the standard curve with the absorption percentages of the standard solution and the semi logarithm values of the AHD 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 AHD 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

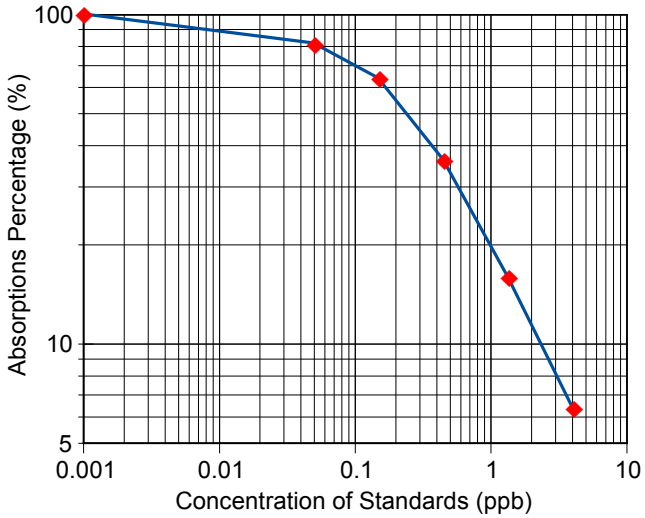
Sensitivity: 0.05 ppb
Detection limit
Tissue and honey·····.1 ppb
Shrimp and fish (some interference in shrimp and fish)·····0.15 ppb
Recovery rate
Shrimp and fish····· ····· 90±15%
Honey, meat /liver····· 80±15%
Cross-reaction rate

AHD	100%	AMOZ	< 0.1%	AOZ	< 0.1%
SEM	0.1%				

Work Sheet of Typical Assay-AHD

Wells	Stds/samples		Mean $A_{450\ nm}$	Absorption Percentage
A1, A2	**Standard A**	0.0 ppb	1.821	100%
B1, B2	**Standard B**	0.05 ppb	1.484	81.49%
C1, C2	**Standard C**	0.15 ppb	1.163	63.87%
D1, D2	**Standard D**	0.45 ppb	0.657	36.08%
E1, E2	**Standard E**	1.35 ppb	0.290	15.93%
F1, F2	**Standard F**	4.05 ppb	0.116	6.37%

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 Aminohydantion (AHD) in the sample. The coupling antigens are pre-coated on the micro-well stripes. The Aminohydantion (AHD) in the sample and the coupling antigens pre-coated on the micro-well stripes compete for the anti-AHD 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 AHD in it. This value is compared to the standard curve and the AHD concentration is subsequently obtained.

MATERIALS AND EQUIPMENT REQUIRED

Equipments: micro plate reader, printer, homogenizer, nitrogen-drying device, vortex, centrifuge, measuring pipettes, and balance (a sensibility reciprocal of 0.01g), single-channel 20 to 200μl and 100 to 1000μl, and multi-channel 250μl.
Reagents: methanol, NaOH, ethyl acetate, N-Hexane, HCl (approx 36.5%), K2HPO4 3H2O, 2-Nitrobenzaldehyde (C7H5NO3), K2Fe(CN)5NO 3H2O and ZnSO4 7H2O

PRECAUTIONS AND SAFETY INSTRUCTIONS

The Aminohydantoin (AHD) ELISA 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 and 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. The 2×concentrated redissolving solution is mixed with deionized water at 1:1 (1 mL concentrated redissolving solution + 1 mL deionized water), used for sample redissolving.
2. *C solution* (for milk sample): dissolve 12.5 g K2Fe(CN)5NO·3H2O in deionized water to 100 mL.
3. *D solution* (for milk sample): dissolve 29.8 g ZnSO4·7H2O in deionized water to 100 mL.
4. 0.1 M K2HPO4: dissolve 22.8 g K2HPO4·3H2O in deionized water to 1 L.
5. 1 M HCl: dissolve 8.6 mL HCl (approx 36.5%) in water to 100 mL.
6. 1 M NaOH: dissolve 4 g NaOH in water to 100 mL.

Samples preparation

a) shrimp, fish and meat

- Homogenize the sample, continue as described in (1-7 **d**).

b) milk

- Put 5 mL milk into centrifuge tube, add C and D solution, 250 µl each.
- Mix thoroughly, use vortex, centrifuge at above 4000 r/min at 4-12 ℃ for 10 min, if centrifuge of constant temperature is not available, chill sample to approx 8 ℃, then centrifuge.
- Continue as described in (1-7 **d**).

C) honey

- Weigh 1 g sample into centrifugal tube.
- Add 4 mL of the deionized water, mix with vortex, then add 0.5 mL 1 M HCl and 100 µL 10 mM 2-Nitrobenzaldehyde solution, mix thoroughly.
- Continue as described in (2-7 **d**).

d) continue as above steps

1. Weigh 1± 0.05 g of the homogenized sample (shrimp, fish or meat), or put 1.1 mL supernatant of centrifuged milk (equivalent to 1 mL milk sample) in a plastic tube, add 4 mL of the deionized water, 0.5 mL 1 M HCl and 100 µl 10mM 2-Nitrobenzaldehyde to each tube, shake properly.
2. Incubate at 37 ℃ over night (approx 16 h).
3. Add 5 mL 0.1 M K_2HPO_4, 0.4 mL 1 M NaOH and 5mL ethyl acetate to each tube, shake vigorously for 5 min.
4. Centrifuge at above 4000 r/min at room temperature (20-25 ℃) for 10 min.
5. Transfer 2.5 mL ethyl acetate (upper layer) into a new centrifugal tube and evaporate to dry by nitrogen or air at 50 ℃.
6. Dissolve the dry residue in 1 mL N-hexane, add 1mL of the diluted redissolving solution, and mix properly, centrifuge at above 4000 r/min at room temperature (20-25 ℃) for 10 min.
7. Take 50 µL of the lower for analysis.

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.

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

1. Bring test kit to room temperature (20-25 ℃) for at least 30 min. Note that each reagent must be shaken to mix evenly before use; put the required micro-well strips into plate frames. Resealed the unused microplate, store at 2-8 ℃, not frozen.
2. 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.

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 50 µL of the antibody working solution into each well, seal the microplate with the cover membrane, and incubate at 37 ℃ for 30 min.
5. Pour liquid out of the wells , flap to dry on absorbent paper, add 250 µL/well of washing buffer to wash microplate for 15 sec, then take out and flap to dry with absorbent paper, repeat 5 times.
6. Add 100 µL enzyme conjugate into each well; and incubate at 37 ℃ for 30 min. Take out microplate, continue as described in step 5.
7. 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;
8. 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).

NOTES:

1. Bring all reagents and micro-well strips to balance at the room temperature (2-25 ℃) 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.
12. The detecting value of the standard solution 1 （0 ppb） of less than 0.5 indicates its degeneration.