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-100320***

Nitroimidazole ELISA KIT

Cat. #DE-100320

For Quantitative Determination of Nitroimidazole in meat/liver/kidney, Fish, shrimp, egg, honey and urine.

India Contact:

Life Technologies (India) Pvt. Ltd.

306, Aggarwal City Mall, Road No. 44, Pitampura, Delhi – 110034, India

Mobile: +91-98105-21400, Tel: +91-11-42208000, 8111, 8222, Fax: +91-11-42208444

Email: customerservice@lifetechindia.com, www.atzlabs.com ; www.lifetechindia.com

Nitroimidazole ELISA KIT Cat. #DE-100320

Kit Components, 96 tests	Cat #
Nitroimidazole coated strip plate (12 strips with 8 removable wells each)	DE-100321
6x standards solution (0.8 ml each): **0.5** ng/mL (yellow cap); **1.5** ng/mL (orange cap); **3** ng/mL(pink cap); **6** ng/mL (purple cap); **12** ng/mL (blue cap); 100 ng/mL (spiking optional red cap)	DE-100322A-F
Nitroimidazole **Negative Control** (white cap); 0.8 mL	DE-100322-NC
Nitroimidazole -HRP Conjugate (100X); Lyophilized **Note**: If the kit is not going to be used for more than a month, store 100X Nitroimidazole-HRP conj. at -20oC	DE-100323
HRP **conjugate Diluent**; 15 mL	DE-100324
Sample Dilution Buffer; 20 mL X 2	100320-SD
20X concentrated **washing buffer** (28 mL)	100320-WB
Stop solution; 14 mL	100320-ST
TMB Substrate solution; 12 mL	100320-TMB
Sample Extraction Buffer conc. 30 g	100320-SEB
Instruction Manual	M-DE-100320

Intended use:

Nitroimidazole ELISA kit is a competitive ELISA for quantitative analysis of Nitroimidazole in meat/liver/kidney, Fish, shrimp, egg, feed, honey, and urine. For in vitro research use only (RUO).

INTRODUCTION:

The term nitroimidazole also refers to a class of antibiotics that share similar chemical structures. 5-Nitroimidazole (O2NC3H2N2H) is the most common isomer of imidazole containing one nitro group. Drugs of the 5-nitro variety include tinidazole, nimorazole, dimetridazole, 6-Amino PA824, ornidazole, megazol, and azanidazole. Drugs based on 2-nitromidazoles include benznidazole, pimonidazole, and metronidazole. nitroimidazoles are a group of drugs that have both antiprotozoal and antibacterial activity. Nitroimidazoles with activity against trichomonads and amebae include metronidazole, tinidazole, nimorazole, flunidazole, and ronidazole. Metronidazole and nimorazole are effective in the treatment of giardiasis, while dimetridazole, ipronidazole, and ronidazole control histomoniasis in poultry. Several nitroimidazoles have activity against trypanosomes. Metronidazole, ronidazole, and other nitroimidazoles are active against anaerobic bacteria.

The use of Nitroimidazole in food-animal production has been banned due to the contribution of antimicrobial-resistant diseases in human populations. Its residue is also very toxic to the nervous system once digested. Alpha Diagnostic Intl's Nitroimidazole ELISA kit is a highly sensitive competitive type assay for the measurement of Nitroimidazole in liver.

Sensitivity (Detection Limit)

Sample Type	Detection Limit (ng/g or ppb)
Meat/Liver/Kidney/Fish	0.5
Shrimp	0.5
Honey/Egg	0.5
Urine	2.5

Sample Type Detection Limit (ng/g or ppb)

Specificity (Cross-Reactivity)

Analytes	Cross-Reactivity (%)
Dimetridazole	100
Metronidazole	100
HydroxyNitroimidazole	32
Ronidazole	14
Ipronidazole	11
Nicarbazin	7
Halofuginone	3
Diclazuril	4
Robenidine	2

Troubleshooting

1. No color in stands-wrong HRP dilution or no HRP or NO TMB added.

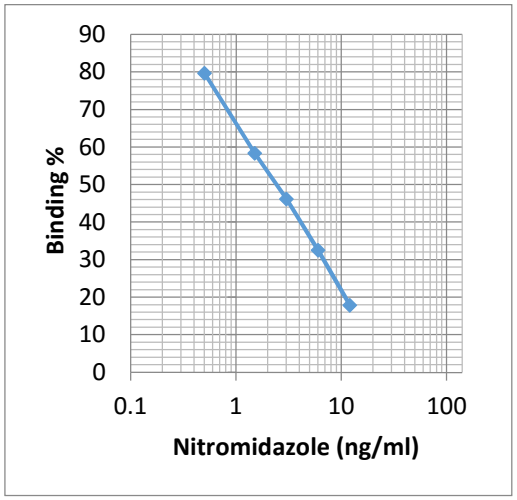
2. Low A450 in Std: usually due to wrong HRP conjugate dilution, cold solutions or cold temp. Check to see the ELISA reader to make sure it is set at 450nm. Blue/yellow color can be visually seen as well.

3. High background or the lowest values of the stds are above 0.5. usually due to contamination of HRP, poor quality of water or more importantly poor washing. Also check HRP conjugate for proper dilution. Too much conjugate will give high background.

Work Sheet of Typical Assay-Nitroimidazole

Wells	Stds/samples	Mean $A_{450\ nm}$	Absorption %
A1, A2	**Negative Control ng/ml**	1.818	100%
B1, B2	**Standard 0.5 ng/ml**	1.450	79.7%
C1, C2	**Standard 1.5 ng/ml**	1.053	58.4%
D1, D2	**Standard 3 ng/ml**	0.839	46.2%
E1, E2	**Standard 6 ng/ml**	0.592	32.5%
F1, F2	**Standard 12 ng/ml**	0.346	17.9%

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.



/6-ADI-ELISA-Arif

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

Nitroimidazole Concentration Calculations

A standard curve can be constructed by plotting the mean relative absorbance (%) obtained from each reference standard against its concentration in ng/mL on a logarithmic curve.

Relative absorbance (%) = 2 $\frac{\text{absorbance standard (or sample)}}{\text{absorbance zero standard}}$ x 100

Use the mean relative absorbance values for each sample to determine the corresponding concentration of the tested drug in ng/mL from the standard curve. A special program with Excel functionality, is available upon request to evaluate the ELISA test results. Please contact your local distributor or service@4adi.com.

PRINCIPLE OF THE TEST

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

MATERIALS AND EQUIPMENT REQUIRED

Equipments: microplate reader (450 nm / 630 nm), vortex, centrifuge, homogenizer, 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: Na_2HPO_4 $12H_2O$, NaH_2PO_4 $2H_2O$.

PRECAUTIONS AND SAFETY INSTRUCTIONS

The Nitroimidazole 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

Be sure samples are properly stored. In general, samples should be refrigerated at 2-4°C for no more than 1-2 days. Freeze samples to a minimum of -20°C if they need to be stored for a longer period. Frozen samples can be thawed at room temps (20 – 25oC / 68 – 77oF) or in a refrigerator before use.

Preparation of 1X Sample Extraction Buffer:

Take all of the powder from the Concentrate of Sample Extraction Buffer bag to a **125-mL bottle, add 90 mL of distilled water**, vortex 2 minutes manually, leave the solution at room temperature for 20 minutes. It is fine if small amount of salt residue is observed at the bottom of the bottle.

Reagent Preparation

IMPORTANT: All reagents should be brought up to room temperature before use (1 – 2 hours at 20 – 25oC / 68 – 77oF); Make sure you read "Warnings and Precautions" section.

Solutions should be prepared just prior to ELISA test. All reagents should be mixed by gently inverting or swirling prior to use. Prepare volumes that are needed for the number of wells being run. Do not return the reagents to the original stock tubes/bottles. Using disposable reservoirs when handling reagents can minimize the risk of contamination and is recommended.

1. Preparation of 100X Nitroimidazole HRP-Conjugate(Powder):

Reconstitute **lyophilized HRP-conjugate** with **250 ul HRP Conjugate Diluent** (DE-100324), mix it well & vortex it gently for 30 seconds, leave it for room temp for **15 mins**. The HRP solution is now **100X**. Store unused **100X HRP conjugate at 4oC for short term use and make aliquots and store frozen at -20 °C** for later use.

Preparation of 1X Nitroimidazole HRP-Conjugate:
Mix 1 volume of **100X** Nitroimidazole-HRP Conjugate with **99 volumes** of HRP Conjugate Diluent.

2. Preparation of 1X Wash Solution

Mix 1 volume of the 20X Wash Solution with 19 volumes of distilled water.

Egg samples

1. Take 2 g of homogenized egg (egg white or yolk, or both) in a tube, add 1 mL of 1x Sample Extraction Buffer, into a centrifugal vial.
2. Add 4 ml of Acetonitrile, vortex for 3 minutes at max. speed manually or 10 min using a multi-tube vortexer. Centrifuge for 5 minutes at 4,000 x g at room temp (20 – 25°C). Take out 1 mL of the supernatant. To another tube into a new vial & use rotary evaporator to dry the sample in a 60-70°C water bath under reduced pressure. Alternatively, the sample can be dried by blowing nitrogen gas in a 60-70°C water bath.
3. Dissolve the dried residue in 0.5 ml of sample dilution buffer and 1 ml of hexane by vortexing at max. speed for 1 minute.
4. Centrifuge for 5 minutes at 4,000 x g at room temp (20 – 25°C). Remove top hexane layer completely Use 50 uL per well for the assay. **Note**: Dilution factor: 1.

Honey

1. Weigh out **2.0 g** of the homogenized sample and add **1 mL** of 1X Sample Extraction Buffer into a centrifugal vial.
2. Add **4 mL** of Acetonitrile, vortex for 3 minutes at maximum speed manually or 10 minutes using a multi-tube vortexer.
3. Centrifuge for **5 minutes at 4,000 x g** at room temperature (20 – 25°C / 68 – 77°F).
4. Transfer **1.0 mL** of the supernatant to another tube (corresponding to 0.5 g of the original sample) into a new vial and use a rotary evaporator to dry the sample in a 60-70°C water bath under reduced pressure. Alternatively, the sample can be dried by blowing nitrogen gas in a 60-70°C water bath.
5. Dissolve the dried residue in **0.5 mL** of Sample Dilution Buffer and **0.5 mL** of hexane by vortexing at maximum speed for 1 minute.
6. Centrifuge for **5 minutes** at 4,000 x g at room temperature (20 – 25□C / 68 – 77□F).
7. Remove top hexane layer completely, use **50 ul** of the bottom layer sample per well for the assay. **Note**: Dilution factor: 1.

Meat/liver/kidney/shrimp/Fish samples

1. Remove fat from the sample. Homogenize the sample with a suitable mixer.
2. Weigh out **2.0 g** of the homogenized sample and add **1 mL** of 1X Sample Extraction Buffer into a centrifugal vial.
3. Add **4 mL** of Acetonitrile, vortex for 3 minutes at maximum speed manually or 10 minutes using a multi-tube vortexer.
4. Centrifuge for **5 minutes at 4,000 x g** at room temperature (20 – 25°C / 68 – 77°F).
5. Transfer **1.0 mL** of the supernatant to another tube (corresponding to 0.5 g of the original sample) into a new vial and use a rotary evaporator to dry the sample in a 60-70°C water bath under reduced pressure. Alternatively, the sample can be dried by blowing nitrogen gas in a 60-70°C water bath.
6. Dissolve the dried residue in **0.5 mL** of Sample Dilution Buffer and **1 mL** of hexane by vortexing at maximum speed for 1 minute.
7. Centrifuge for 5 minutes at 4,000 x g at room temperature (20 – 25□C / 68 – 77□F).
8. Remove top hexane layer completely, use **50 ul** of the bottom layer sample per well for the assay. **Note**: Dilution factor: 1.

Urine/Serum samples:

1. Centrifuge 0.5 mL of urine or serum at 4,000 x *g* for 5 minutes.
2. Dilute the supernatant with 1X Sample Extraction Buffer (1:4), (For example, 100 ul of supernatant + 400 ul of Buffer). Take 50 ul of diluted sample per well for the assay.

Note: Dilution factor: 5. (*to reduce the background you can dilute the sample with 1X sample dilution buffer (1:9) (e.g; 20 ul of supernatant + 80 ul of buffer*). Dilution factor: 10.

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).

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. Take the required micro-well strips and plate frames. Re-sealed the unused microplate, store at 2-8℃, not frozen. **Prepare 1X wash buffer and 1x HRP-conjugate.**

Label the individual strips that will be used and aliquot reagents as the following example:

Component	Volume per Reaction	24 Reactions
1X Nitroimidazole HRP-Conjugate	100 ul	2.4 ml
1X Wash Solution	1 ml	24 ml
Stop Buffer	100 ul	2.4 ml
TMB Substrate	100 ul	2.4 ml

ELISA Test procedure:

1. Add **50 uL** of each Standards in duplicate into different wells (F Add standards to plate only in the order from low concentration to high concentration).

2. Add **50 uL** of each sample in duplicate into different sample wells.

3. Add **100 uL of 1x HRP Conjugate** and mix well by gently rocking the plate manually for 1 minute.

4. Incubate the plate for **45 minutes** at room temperature (20 – 25°C / 68 – 77°F).

5. Wash the plate **3 times with 250 uL** of 1X Wash Solution. After the last wash, invert the plate and gently tap the plate dry on paper towels (F Perform the next step immediately after plate washings. Do not allow the plate to air dry between working steps).

6. Add **100uL** of TMB substrate. Time the reaction immediately after adding the substrate. Mix the solution by gently rocking the plate manually for 1 minute while incubating.

7. After incubating for **30 minutes** at room temperature (20 – 25°C / 68 – 77°F), add 100 uL of Stop Buffer to stop the enzyme reaction. Read the plate as soon as possible following the addition of Stop Buffer on a plate reader with 450 nm wavelength.