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-100200***

Gentamicin ELISA KIT

Cat. #DE-100200

For Qualitative and Quantitative Determination of Gentamicin in chicken and liver.

India Contact:

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

Gentamicin ELISA KIT Cat. #DE-100200

Kit Components, 96 tests	Cat #
Micro-well coated strip plate (12 strips with 8 removable wells each)	DE-100201
6x standards solution (0.8 ml each): 0.25 ng/mL (yellow cap); 0.75 ng/mL (orange cap); 1.5 ng/mL(pink cap); 3.0 ng/mL (purple cap); 15 ng/mL (blue cap); 50 ng/mL (spiking optional red cap)	DE-100202
Gentamicin negative control (white cap) 0.8 mL	DE-100202NC
Gentamicin Antibody #1 (12 mL)	DE-100204
Enzyme conjugate(**100X**) Antibody#2 (250 ul)	DE-100203
Antibody Diluent #2 (20 mL)	DE-SSA
10X Sample Extraction Buffer (25 mL)	DE-SB
20X concentrated washing buffer (28 mL)	DE-WB
Stop solution (14 mL)	DE-ST
TMB Substrate Solution (12 mL)	DE-TMB
Milk Clean Up Reagent (optional,10 ml)	DE-MR
Milk Balance Buffer (optional ,1.6 ml)	DE-MB
Instruction Manual	M-DE-100200
Notes: if the **antibody #1** and **100XHRP-conjugate** will not be used for 1 month or more, store at **-20oC** in a suitable aliquots.	

Gentamicin ELISA Test Kit is a competitive enzyme immunoassay for the quantitative analysis of Gentamicin in egg, embryonic egg, feed, honey, meat/liver/kidney, milk, serum, Soybean Casein Digest Broth and urine. For in vitro research use only.

INTRODUCTION

Gentamicin is an antibiotic from the group aminoglycoside. Aminoglycoside is a molecule composed of a sugar and amino group. Gentamicin is used to treat various types of bacterial infections mainly the ones caused by gram-negative bacteria. Gentamicin has a unique characteristic for being an antibiotic; it is very high heat resistance and will remain stable and active even after being autoclaved. This characteristic is very useful in the cell culture field for the preparation of growth media.

Gentamicin is produced by Micromonospora through fermentation sites mostly located in China, and South Korea. Micromonospora is a type of bacteria from the family Micromonosporaceae. Micromonospora lives in water and soil. It is a spore-forming, aerobic gram-positive bacterium. Gentamicin is a bactericidal antibiotic that works by binding the 30S subunit of the bacterial ribosome interfering protein synthesis. Gentamicin can damage the hair cells of the inner ear and maybe even the kidney, leading to permanent bilateral vestibulopathy and temporally acute renal failure. Gentamicin is usually used to treat serious, life threatening infections. Symptoms may include; balance difficulty, bouncing/unsteady vision, ringing in the ears, difficulty multi-tasking principally standing up, nausea, mental confusion, tiredness, kidney damage, and difficulty with short-term memory. Gentamicin is usually applied to the patient through IV (intravenous) as an antibiotic without the explanation of severe and permanent potential consequence.

The European Commission established a gentamicin Maximum Residue Limit (MRL) of 50ppb for muscle and fat, 200ppb for liver, 750 ppb for kidney and 100ppb for milk. Gentamicin ELISA Test Kit enables international and government regulatory agencies, food manufacturers and processors, as well as quality assurance organizations, to detect Gentamicin in various sample types and to satisfy customer concerns about food safety. The unique features of the kit are high recovery (80%), rapid sample preparation (10-40 mins), high sensitivity (0.25 ng/g or ppb), rapid test (< 2 hrs). This kit can be used in a variety of samples (food, meat, plasma etc).

Technical specifications

Sensitivity (Detection Limit)

Sample Type Detection Limit	(ng/g or ppb)
Embryonic Egg	4.0
Egg	5.0
Feed	6.25
Honey	6.25
Meat/liver/kidney	2.5
Milk	0.25
Soybean Casein Digest Broth (SCD Broth)	12.5
Urine, Serum	5.0

Specificity (Cross-Reactivity)

Analytes Cross-Reactivity	(%)
Gentamicin	100
Neomycin	<0.1
Kanomycin	<0.1
Streptomycin	<0.1
Tobramycin	<0.1
Dihydrostreptomycin	<0.1

Troubleshooting

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

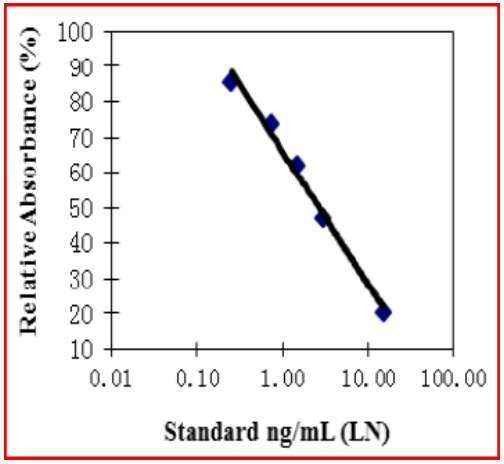
2. Low A450 in Std: usually due to wrong HRP conjugate dilution, cold solutions or cold temp. Check to see the ELISA reader to make sure it is set at 450nm. Blue/yellow color can be visually seen as well.

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-Gentamicin

Wells	Stds/samples	Mean $A_{450\ nm}$	Absorption Percentage
A1, A2	**Standard A 0 ng/ml**	2.04	
B1, B2	**Standard B 0.25 ng/ml**	1.88	
C1, C2	**Standard C 0.75 ng/ml**	1.64	
D1, D2	**Standard D 1.5 ng/ml**	1.45	
E1, E2	**Standard E 3.0 ng/ml**	1.16	
F1, F2	**Standard F 15 ng/ml**	0.54	

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)

Gentamicin 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 (%) = $\frac{\text{absorbance standard (or sample)}}{\text{absorbance zero standard}} \times 100$

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 .

The use of gentamicin 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 Gentamicin ELISA kit is a highly sensitive competitive type assay for the measurement of Gentamicin in chicken and liver.

PRINCIPLE OF THE TEST

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

1. **Preparation of 1X Sample Extraction Buffer:**
Mix 1 volume of 10X Sample Extraction Buffer with 9 volumes of distilled water.

2. **Preparation of 1X Embryonic Egg Buffer:**
Mix 1 volume of 8X Embryonic Egg Buffer with 7 volumes of distilled water.

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 1X HRP-Conjugated Antibody #2
Mix 1 volume of 100X HRP-Conjugated Antibody #2 with 99 volumes of Antibody #2 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 0.5 g of homogenized egg (egg white or yolk, or both) in a tube, add 9.5 mL of 1x Sample Extraction Buffer, mix well by vortexing.
2. Centrifuge the sample at 2,000 x g for 5 minutes. Take out 1 mL of the supernatant. Use 50 ul per well for the assay. **Note**: Dilution factor: 20.

Embryonic Egg samples

1. Take 0.5 g of homogenized embryonic egg in a tube, add 2 mL of 1x Embryonic Egg Buffer, mix well by vortexing 3 minutes.
2. Centrifuge the sample at 4,000 x g for 10 minutes. Take out 0.5 mL of the supernatant (avoid the top fat layer!), add 1 mL of hexane, vortex for 2 minute.
3. Centrifuge the sample at 10,000 x g for 4 minutes. Remove the top hexane layer completely, transfer 250 uL of lower layer to a new tube, add 250 uL of 1X Sample Extraction Buffer, mix well. Use 50 uL per well for the assay. **Note**: Dilution factor: 10.

Feed samples

1. Homogenize the feed sample with a suitable mixer.
2. Weigh out 1.0 g of the homogenized sample and add 5 mL of 3% trichloracetic acid.
3. Homogenize for 1 minute. Place the sample on a rotating wheel or shaker for 30 minutes. Cool the sample on ice or at 4oC, then centrifuge for 5 minutes at 2,000 x g.
4. Dilute 200 uL of the clear supernatant (in case it is not clear, recentrifuge or filtrate with Whatman No.1 filter paper) with 800 uL of 1X Sample Extraction Buffer; adjust pH to 7.4 with about 25 uL of 1M NaOH. Take 50 uL of the diluted sample per well for the assay.
Note: Dilution factor: 25.

Honey

1. Take 1 g of homogenized honey in a tube, add 4 mL of 1x Sample Extraction Buffer, mix well by vortexing. Centrifuge the sample at 2,000 x g for 5 minutes. Take out 1 mL of the supernatant, add 4 mL of 1x Sample Extraction Buffer, mix well by vortexing.
2. Use 50 uL per well for the assay. **Note**: Dilution factor: 25.

Meat/liver/kidney samples

1. Remove fat from the sample. Homogenize the sample with a suitable mixer. Weigh out 1.0 g of the homogenized sample and add 4 mL of 3% trichloracetic acid. Homogenize for 1 minute.
2. Place the sample on a rotating wheel or shaker for 30 minutes. Cool the sample on ice or at 4oC, then centrifuge for 5 minutes at 2,000 x g.
3. Dilute 200 uL of the clear supernatant (in case it is not clear, recentrifuge or filtrate with Whatman No.1 filter paper) with 200 uL of 1X Sample Extraction Buffer; adjust pH to 7.4 with about 20 uL of 1M NaOH. Take 50 uL of the diluted sample per well for the assay.
Note: Dilution factor: 10.

Milk samples

1. Add 1 mL of milk sample into a tube. Add 100 uL of Milk Clean Up Reagent to each sample.
2. Vortex vigorously for 20 seconds. Centrifuge at 4,000 x *g* for 10 minutes.
4. Take 200 uL of supernatant and add 11 uL of 1X Milk Balance Buffer, mix well.
5. Use 50 uL of the sample for the assay. ***Note:*** *Dilution factor: 1.2 .If gentamicin concentration is expected high, the sample obtained from Step 4 can be further diluted with 1X Sample Extraction Buffer.*

Soybean Casein Digest Broth (SCD Broth)

1. Transfer 60 ul of the SCD broth sample to 2.94 mL of 1X Sample Extraction Buffer.
2. Vortex 30 seconds. Use 50 uL per well for the assay. ***Note:*** *Dilution factor: 50.*

Urine/Serum samples

1. Centrifuge 0.5 mL of urine or serum at 2,000 x *g* for 5 minutes.
2. Recover the supernatant and dilute it 20 fold with 1x Sample Extraction Buffer (For example, add 25 uL sample to 475 uL 1X Sample Extraction Buffer).
3. Use 50 uL per well for the assay. ***Note:*** *Dilution factor: 20.*

a) Kidney/Meat/Liver samples

1. Remove fat from sample, homogenize the sample with a suitable mixer, Take 1.0 g of homogenize sample & add 4 mL of trichloracetic acid, homogenize for 1 min, place the sample on a shaker for 30 min.
2. Cool sample on ice or 4 □ then centrifuge at above 2000 g at room temperature (20-25 □) for 5 min
3. Dilute **200 µL** of supernatant (upper layer), add 200 µL of 1X Sample Extraction buffer, adjust pH to 7.4 with about 20 ul of 1M NaOH, mix properly.
4. Take 50 µL for analysis. **Note**: **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
Gentamicin Antibody #1	100 ul	2.4 ml
1X HRP-Conjugated Antibody #2	150 ul	3.6 ml
1X Wash Solution 2.0 mL 48 mL	2.0 ml	48 ml
Stop Buffer	100 ul	2.4 ml
TMB Substrate	100 ul	2.4 ml

ELISA Test procedure

1. .Add **50 µL** of the sample or standard solution to separate duplicate wells, add **100 µL** of the antibody solution#1 into each well. Vortex evenly, seal the microplate with the cover membrane, and incubate at room temp 25-28 ℃ for **30 minutes.**

2. Pour liquid out of the microwells, add **250 µL**/well of washing buffer for 10 sec, repeat **three times**, then flap to dry with absorbent paper (if there are the bubbles after flapping, cut them with the clean tips).

3. Add **150 µL** of **enzyme antibody solution#2** into each well. Gently mix the well contents manually for 5-10 seconds. cover the plates, and incubate for **30 minutes at room temp (**at 25-28 ℃)

4. Wash the plate three times with 250 ul/well same as in step#2 above.

5. Add **100 µL** of **TMB substrate** solution into each well. Mix gently by shaking the plate manually, and incubate at room temp for 15 min. Blue color develops in standards and samples.

6. Add **100 µL** of the **stop solution** into each well, Mix gently by shaking the plate manually. Blue color turns yellow. Read the plate at A450 nm (or dual filters using 630nm as reference) to determine the OD value within 5 min.