

Antibiotics ELISA kits available from ADI:

http://4adi.com/commerce/catalog/spcategory.jsp?category_id=2739

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	Salbutamol 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	Nitrofurantoin (AMOZ) ELISA kit (For Fish, Shrimp, Honey, Chicken/Liver), 96 tests
DE-100070	Nitrofurantoin (AHD) ELISA kit, (For Fish, Shrimp, Honey, Chicken/Liver), 96 tests
DE-100075	Nitrofurantoin (SEM) ELISA kit (Honey, Fish, Shrimp, Chicken/Liver, Fish/Shrimp), 96 tests
DE-100080	Nitrofurantoin (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-100130	Enrofloxacin ELISA kit, 96 tests (Chicken/Liver, Honey, Milk)
DE-100230	Olaquinox 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-100160

Tylosin ELISA KIT

Cat. #DE-100160, 96 Tests

For Qualitative and Quantitative Determination of Tylosin in feed, meat, liver and honey samples



For In Vitro Research Use Only



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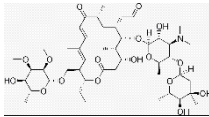
Tylosin ELISA KIT Cat. #DE-100160, 96 tests

Kit Components, 96 tests	Qty
Micro-well coated strip plate (12 strips with 8 removable wells each), # 100161P	1
4x standard solution (0.6 ml each): 0 ng/ml, 0.75 ng/ml, 3.5 ng/ml, 20 ng/ml, #100162A-D	4 Vials
Tylosin-AP conjugate (10.5 mL) #100163	1 Bottle
Tylosin Substrate solution, 10.5 ml ready-to-use #100164	1 Bottle
Stop solution, 5.5 ml, #100160ST	1 Bottle
Wash buffer (10X), 15 mL, #100160WB	1 Bottle
Instruction Manual, #M-100160	1

Intended use:

ADI's Tylosin ELISA kit is a highly sensitive competitive type assay for the measurement of Tylosin in samples. For research use only (RUO), not for diagnosis, cure or prevention of the disease..

INTRODUCTION



Tylosin (C₄₆H₇₇NO₁₇, mol wt 916.1) is used by veterinary medicine as a macrolide antibiotic. Other examples of macrolide antibiotics are erythromycin, clarithromycin, azithromycin, spiramycin and dirithromycin.

Tylosin works against gram positive organisms and a few gram negative organisms. It is an outcome of the fermentation of the microorganism *Streptomyces fradiae*. Tylosin is licensed to use for antibiotic treatment of infections in live stock. In small animals it is used as an anti-inflammatory to treat colitis and to reduce epiphora around the eyes of white faced dogs. It can also be used in cattle to treat respiratory infections, metritis and mastitis. It will also treat mastitis in sheep and goats. In swine it is used to treat enteritis, pneumonia, erysipelas and infectious arthritis. Besides using as a treatment drug it is also used as a growth promoter for some terrestrial and aquatic food production animals.

It works by binding to the 50S subunit of the bacterial ribosome and leads to inhibition the synthesis of protein. Tylosin will work against *Staphylococci*, *Streptococci*, *Corynebacteria*, *Erysipelothrix*, *Campylobacter coli* and some spirochaetes. It will also work against *Mycoplasma* species isolated from both mammalian and avian hosts.

MIC susceptibility data for a few medically significant pathogens:

Mycoplasma bovis: 0.06 - 4 µg/ml
Staphylococcus aureus: 0.5 - >128 µg/ml

Technical specifications

Sensitivity: 0.005 ng/ml

Detection limit

Meat, liver.....0.1 ppb
 Honey.....0.05 ppb

Recovery rate

Meat.....90%±15%
 Liver.....80%±10%
 Honey.....85%±15%

Cross-reactivity

following compounds tested at the stated levels are found to give results not greater than a level of 2.5 ng/mL of tylosin.

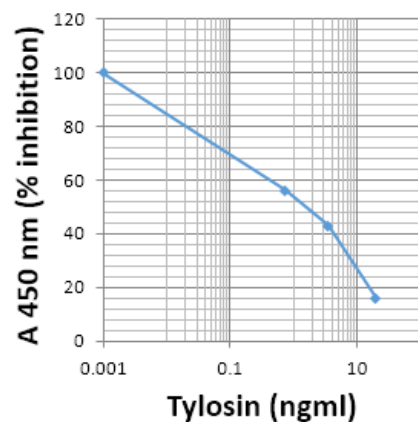
Compound	Conc. (ng/mL)	% Inhibition
Gentamicin	10,000	<10
Neomycin	10,000	<10
Monensin	10,000	<10
Sulfamethazine	10,000	<10
Sulfadimethoxine	10,000	<10
Zearalenone	10,000	<10
Narasin	10,000	<10

Other substances and/or factors not listed above may interfere with the test.

Work Sheet of Typical Assay-Tylosin

Wells	Stds/samples	Mean A ₄₅₀ nm	Absorption Percentage
A1, A2	Standard A 0 ng/ml	1.80	100
B1, B2	Standard B 0.75 ng/ml	1.01	56
C1, C2	Standard C 3.5 ng/ml	0.77	43
D1, D2	Standard D 20 ng/ml	0.28	16

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.



7-arif/DE-100160-Tylosin-ELISA-Graph
A typical assay Std Curve (do not use this for calculating sample values)

1. Calculation of results

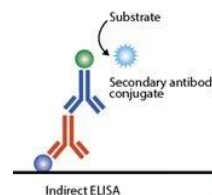
(a) Average the A₄₅₀ (ODs) for each standard concentration of tylosin including 0 ng/mL (OD₀).

(b) calculate % of Inhibition
= $100 - (ODs / OD_0) \times 100$

2. Plot values of % of Inhibition, step 1 (b), against their corresponding concentrations on Log₁₀ paper.

3. Calculate tylosin concentration in the sample by interpolation and multiply by the sample's dilution factor to obtain the actual quantity of tylosin

PRINCIPLE OF THE TEST



Indirect ELISA

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

subsequently obtained.

MATERIALS AND EQUIPMENT REQUIRED

Equipments: microplate reader (450 nm/620 nm), homogenizer, oscillator, vortex, centrifuge, and balance (a sensibility reciprocal of 0.01 g)

Micropipettors: single-channel 20-1000 μ L, and multi-channel 250 μ L.

Reagents: Ethanenitrile (CH₃CN), Methanol, deionized water, 0.1 M HCl, NaOH, CHCl₃

Solution preparation for sample pre-treatment

- 0.1 M NaOH:** dissolve 0.4 g NaOH in deionized water to 100 mL.
- Sample extracting solution:** mix 84 mL Acetonitrile and 16 mL 0.1 M HCl, then add 18 mL methanol, mix evenly.
- The redissolving buffer (PBS, pH 7.4).

PRECAUTIONS AND SAFETY INSTRUCTIONS

The Tylosin Kit is for research use only.

Stop Solution contains diluted 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 website.

SAMPLE PRE-TREATMENT

Instructions

The following points must be dealt with before the pre-treatment of any kind of sample:

- Only the disposable tips can be used for the experiments and the tips must be changed when used for absorbing different reagents.
- 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.

Samples preparation

a) Animal tissue liver and meat sample

1. Homogenize the sample.
2. Take 2 ± 0.05 g of the homogenized sample into 50 mL tube, add 8 mL of Sample extracting solution, shake properly with oscillator for 5 min.
3. Centrifuge at above 3000 r/min at room temperature (20 - 25 °C) for 10 min.
4. Take 2 mL supernatant, add 1 mL of 0.1 M NaOH, mix properly, add 3 mL CHCl₃ to extract, shake with oscillator for 5 min.
5. Centrifuge at above 3000 r/min at room temperature (20-25 °C) for 10 min, remove the upper layer, take the lower, blow to dry with nitrogen.
6. Dissolve the dry residues in 1 mL of the diluted redissolving solution.
7. Take 50 µL for analysis.

Fold of dilution of the sample: 2

b) Honey

1. Weigh 1 ± 0.05 g sample into 50 mL centrifuge tube, add 2 mL deionized water, vortex for 2 min to dissolve, then add 10 mL CHCl₃, shake upside and down for 5 min.
2. Centrifuge at above 3000 r/min at room temperature (20 – 25 °C) for 10 min, remove the upper layer, take organic phase (the lower), blow to dry with nitrogen.
3. Dissolve the dry residue in 1 mL of the diluted redissolving solution.
4. Take 50 µL for analysis.

Fold of dilution of the sample: 1

STORAGE AND STABILITY

Storage: store at 2 to 8 °C, not frozen.

Expiration date: 12 months; date of production is on box.

Reagent Preparation

1. Dilute wash buffer conc (10X) 1:10 in distilled water (dilute 15 mL of the 10x concentrated washing buffer with the distilled or deionized water to 140 mL). Store unused 1X conjugate at 2-4°C.

ELISA Assay Procedure

1. Bring all reagents and micro-well strips to the room temperature (20-25°C);
2. Return all reagents to 2-8°C 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. Number the micro-wells according to samples and standard solution; each sample and standard solution should be performed in duplicate; record their positions.
2. Add **25 µL standard solution and sample** to separate duplicate wells, and **add 100 µL enzyme conjugate** into each well. Mix gently by shaking the plate manually, cover the microplate with the cover membrane, and **incubate at room temp. for 40 min.**
3. Pour liquid out of microwell, add **250 µL/well of washing buffer for 10 sec, repeat four to three times**, then flap to dry (if there are the bubbles after flapping, cut them with the clean tips).
4. **Add 100 µL of the substrate solution** into each well. Mix gently by shaking the plate manually, cover the plate and **incubate at room temp for 20 min in the dark.** Yellow color appears in standards and samples.
5. Add **50 µL of the stop solution** into each well. Mix gently by shaking the plate manually (color remains yellow). Read the plate 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 °C or the temperature of the reagents and the samples being not returned to the room temperature (20-25 °C) 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 diluted 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 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. 20 min for coloration after addition of substrate A and B, if the coloration is light, prolong time and don't exceed 30 min.
9. The optimum reaction temperature is 37 °C, and too high or too low temperatures will result in the changes in the detecting sensitivity and OD values.