Tylosin ELISA Kit

Catalog Number KA1427

96 assays

Version: 06

Intended for research use only

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Introduction

Background

Tylosin (MW = 916.14), is a macrolide antibiotic that is active against gram-positive and gram-negative bacteria. It is also effective in treating respiratory diseases caused by mycoplasms. It is widely used as a feed additive for growth promotion as well as therapeutically in food producing animals, including cattle, chickens and swine. Tylosin is also used for the reduction of the incidence of liver abscesses caused by sphaerophorous necrophorous. Food and Drug Administration of the United States has set the guidelines for the allowable level of tylosin in animal feeds and the specific tolerance for tylosin in edible products of animals. The Tylosin ELISA kit is a rapid and simple detection system for tylosin in feed extract.

Principle of the Assay

The enzyme immunoassay for tylosin is based on the competition between the tylosin to be assayed and the tylosin-alkaline phosphatase conjugate, for binding to rabbit antibody directed against tylosin, coated onto microwells. The sample containing the tylosin, and the tylosin-alkaline phosphatase conjugate, when added to the microtiter wells, compete for binding to a limiting number of antibody sites. After incubation, each well is rinsed in order to remove non-bound components. The bound enzymatic activity is then measured by the addition of a chromogenic substrate. The intensity of the color developed is inversely proportional to the concentration of tylosin in the sample. The concentration is calculated on the basis of a standard curve.

General Information

Materials Supplied

List of component

Component	Amount
96-wells microtiter plate (#S). Twelve strips of 8 detachable wells, coated with Anti-Tylosin antibody.	96-wells (12 x 8)
Calibrators containing 0, 0.75, 3.5, 20 ng/mL of Tylosin.	0.6 mL x 4
Tylosin-Alkaline phosphatase Conjugate (TLS-ALP) (#3).	10.5 mL
p-nitrophenyl phosphate (pNPP) substrate (#5). Ready to use.	10.5 mL
Wash Buffer (10xPBS-Tween) (#6). Dilute 10 fold with distilled or deionized water to 150 mL prior to use.	15 mL
Stop Solution, 3 N NaOH (#7).	5.5 mL

Storage Instruction

All reagents of the kit are stable, if stored at 2-8 °C, until the expiration date stated on the kit.

Materials Required but Not Supplied

- ✓ Pipettors capable of delivering 25 µL, 50 µL and 100 µL.
- ✓ Microtiter plate reader (wavelength 405 nm).
- ✓ Plate washer or squeezable wash bottle.
- ✓ Timer.
- ✓ Absorbent paper towels.

Precautions for Use

- ✓ Do not mix reagents from different lots.
- ✓ If concentrations of tylosin in the samples are high (>25 ng/mL), dilute sample such that points fall in the middle range of the standard curve.
- ✓ Do not return unused reagents back into their original bottles.
- ✓ Samples tested should have a pH of 7.0 (± 1.0). Excessive alkaline or acidic conditions may affect the test results.
- ✓ The stop solution contains NaOH. Avoid contact with skin or eyes. If exposed, flush with water.
- ✓ Dispose of all materials, containers and devices in the appropriate receptacle after use.

Assay Protocol

Assay Procedure

Let the components of the kit equilibrate to room temperature before use.

1. Carefully add 25 µL of standard or samples to the bottom of each well. Slightly tap the side of the strip holder to evenly distribute the sample.
2. Avoid touching the well with pipette tip and add 100 µL of TLS-ALP conjugate (#3) to each well. Slightly tap the side of the strip holder to properly mix the sample and enzyme conjugate.
3. Incubate at room temperature for 40 minutes.
4. After incubation, dispose the solution in the wells by inverting and shaking. Wash microtiter wells 3 times with wash buffer to remove the non-bound conjugate. Washing may be done manually as follows: use squeeze bottle to fill wells gently with wash buffer, dumping the wells between each wash by inverting and shaking. After the third wash, tamp holder with washed strips onto a piece of absorbent paper.
5. Add 100 µL of pNPP substrate (#5) to each well and incubate at room temperature for 20 min. To avoid contamination, place the needed amount of substrate into a test tube and dispense only from the tube itself.
6. Add 50 µL of Stop Solution (#7) to each well and tap the strip holder for proper mixing.
7. Read absorbance at 405 nm using an ELISA reader.

✓ Simplified Assay Procedure

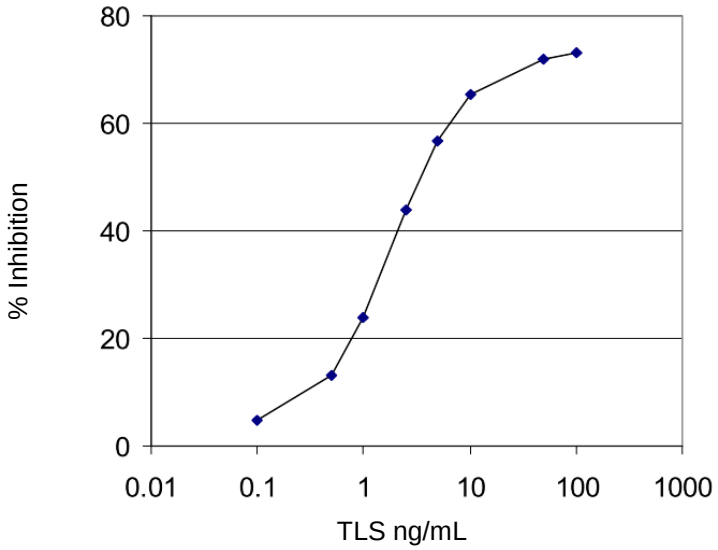
1. Add sample or standard (25 µL).
2. Add Enzyme conjugate (100 µL). 40 min at RT.
3. Wash 3x.
4. Add pNPP (100 µL), wait for 20 min. at RT.
5. Add stopping solution (50 µL) and read at 405 nm.

Data Analysis

Calculation of Results

1. Calculation
   (a) Average the absorbance (OD_s) for each standard concentration of tylosin including 0 ng/mL (OD_0).
   (b) % of Inhibition = 100 - (OD_s / OD_0) x 100
2. Plot values of % of Inhibition, step 1 (b), against their corresponding concentrations on Log_{10} 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.

Tylosin Inhibition curve



Performance Characteristics

✓ Cross Reactivity

By the assay, the 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

There is the possibility that other substances and/or factors not listed above may interfere with the test.

Resources

Plate Layout

12								
11								
10								
9								
8								
7								
6								
5								
4								
3								
2								
1								
	A	B	C	D	E	F	G	H