# Tylosin ELISA Kit (OKAO00132)
## Instruction for Use

For the quantitative detection of Tylosin (SDM) concentration in tissue (chicken, duck, porcine meat, beef and mutton), liver (chicken, duck, porcine meat, beef and mutton), honey.

This product is intended for research use only.

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# 1. Background

## Principle

Aviva Systems Biology Tylosin ELISA Kit (OKAO00132) is based on standard competitive inhibition enzyme-linked immuno-sorbent assay technology. Tylosin has been pre-coated onto a 96-wellplate (12 x 8 Well Strips). Standards or test samples are added to the wells along with a rabbit anti-Tylosin antibody and an anti-rabbit / HRP Conjugated antibody, then incubated. Wells are washed and detection substrates are added and incubated. An enzymatic reaction is produced through the addition of TMB which is catalyzed by HRP generating a blue color product that changes yellow after adding acidic stop solution. The density of yellow coloration read by absorbance at 450 nm which is quantitatively proportional to the amount of detector antibody bound in the well and inversely proportional to the amount of Tylosin in the sample.

## Background

Tylosin is a bacteriostat feed additive used in veterinary medicine. It has a broad spectrum of activity against Gram-positive organisms and a limited range of Gram-negative organisms. It is found naturally as a fermentation product of Streptomyces fradiae. It is a macrolide antibiotic. Tylosin is used in veterinary medicine to treat bacterial infections in a wide range of species and has a high margin of safety. It has also been used as a growth promotant in some species, and as a treatment for colitis in companion animals. Like other macrolides, tylosin has a bacteriostatic effect on susceptible organisms, caused by inhibition of protein synthesis through binding to the 50S subunit of the bacterial ribosome.

## General Specifications

| General Specifications | |
|---|---|
| Specificity | Tylosin<br>CAS#: 1401-69-0<br>PubChem#: 12873460<br>Alias: TYL; Tylosin; 1401-69-0; AKOS016010155; AK115101 |
| Detection Range | 1.5-121.5 ppb |
| Limit of Detection | 1.5 ppb |

# 2. Storage and Stability

• Upon receipt store kit at 4°C. Do not use past expiration date.
• The stability of ELISA kit is determined by the loss rate of activity. The loss rate of this kit is less than 5% within the expiration date under appropriate storage condition.

## 3. Kit Components

•The following reagents are the provided contents of the kit.

| Description | Quantity | Storage Conditions |
|---|---|---|
| Tylosin Microplate | 96 Wells (12 x 8 Well | 4°C<br>Do not use past expiration date |
| Tylosin Standards | 6 x 1 mL | |
| Tylosin Rabbit Detector Antibody | 1 x 7 mL | |
| Anti-Rabbit / HRP Conjugate | 1 x 7 mL | |
| Detection Reagent A | 1 x 7 mL | |
| Detection Reagent B | 1 x 7 mL | |
| 2X Sample Prep Buffer | 2 x 20 mL | |
| 20X Wash Buffer | 2 x 20 mL | |
| Stop Solution | 1 x 7 mL | |

## 4. Required Materials Not Supplied

- Microplate reader capable of reading absorbance at 450 nm.
- Automated plate washer (optional).
- Pipettes capable of precisely dispensing 0.5 µL through 1 mL volumes of aqueous solutions.
- Pipettes or volumetric glassware capable of precisely measuring 1 mL through 100 mL of aqueous solutions.
- New, clean tubes and/or micro-centrifuge tubes for the preparation of standards or samples.
- Absorbent paper or paper toweling.
- Distilled or deionized ultrapure water.
- Heater water bath
- Centrifuge
- Reagents: NaOH, HCl (approximately 36.5%), Acetonitrile ($CH_3CN$), Methanol, Trichloromethane.

## 5. Precautions

- Read instructions fully prior to beginning use of the assay kit.
- Any deviations or modifications from the described method or use of other reagents could result in a reduction of performance.
- Reduce exposure to potentially harmful substances by wearing personal protective lab equipment including lab coats, gloves and glasses.
- For information on hazardous substances included in the kit please refer to the Material Safety Data Sheet (MSDS).
- Kit cannot be used beyond the expiration date on the label.

## 6. Technical Application Tips

• Do not mix or substitute components from other kits.

• To ensure the validity of experimental operation, it is recommended that pilot experiments using standards and a small selection of sample dilutions to ensure optimal dilution range for quantitation.

• Samples exhibiting OD measurements higher than the highest standard should be diluted further in the appropriate sample dilution buffers.

• Prior to using the kit, briefly spin component tubes to collect all reagents at the bottom.

• Replicate wells are recommended for standards and samples.

• Cover microplate while incubating to prevent evaporation.

• Do not allow the microplate wells dry at any point during the assay procedure.

• Do not reuse tips or tube to prevent cross contamination.

• Avoid causing bubbles or foaming when pipetting, mixing or reconstituting.

• Completely remove of all liquids when washing to prevent cross contamination.

• Prepare reagents immediately prior to use and do not store, with the exception of the top standard.

• Equilibrate all materials to ambient room temperature prior to use (standards exception).

• For optimal results for inter- and intra-assay consistency, equilibrate all materials to 37°C prior to performing assay (standards exception) and perform all incubations at 37°C.

• Pipetting less than 1 µL is not recommended for optimal assay accuracy.

• Once the procedure has been started, all steps should be completed without interruption. Ensure that all reagents, materials and devices are ready at the appropriate time.

• Incubation times will affect results. All wells should be handled in the same sequential order and time intervals for optimal results.

• Samples containing precipitates, fibrin strands or bilirubin, or are hemolytic or lipemic might cause inaccurate results due to interfering factors.

• TMB Substrate is easily contaminated and should be colorless or light blue until added to plate. Handle carefully and protect from light.

• To minimize influences on the assay performance, operation procedures and lab conditions, especially room temperature, air humidity, incubator temperature should be strictly controlled. It is also strongly suggested that the whole assay is performed by the same operator from the beginning to the end.

# 7. Reagent Preparation

• Equilibrate all materials to room temperature prior to use and use prepare immediately prior to use.

## 7.1 0.1 M NaOH

7.1.1 Prepare 100 mL of fresh **0.1 M NaOH** by adding 400 mg of NaOH to 100 mL of ultrapure water immediately prior to use.

7.1.2 Mix gently and thoroughly.

7.1.3 Do not store for future use.

## 7.2 0.1 M HCl

7.2.1 Prepare 100 mL of fresh **0.1 M HCl** immediately prior to use.

7.2.2 Add 860 µL HCl (36%) to 100 mL ultrapure water.

7.2.3 Mix gently and thoroughly.

7.2.4 Do not store for future use.

## 7.3 1X Anti-Rabbit / HRP Conjugate

7.3.1 Prepare the **1X Anti-Rabbit / HRP Conjugate** immediately prior to use by diluting the 10**X Avidin-HRP Conjugate** 1:10 with **Conjugate Diluent**.

7.3.2 For each well strip to be used in the experiment (8-wells) prepare 500 µL by adding 50 µL of **10X Avidin-HRP Conjugate** to 450 µL **Conjugate Diluent**.

7.3.3 Mix thoroughly and gently. Hold no longer than 2 hours prior to using in procedure. Do not store at 1X concentration for future use.

## 7.4 Microplate Preparation

•Micro-plates are provided ready to use and do not require rinsing or blocking.

•Unused well strips should be returned to the original packaging, sealed and stored at °4C.

•Equilibrate microplates to ambient temperatures prior to opening to reduce potential condensation.

# 8. Sample Preparation

## 8.1 Sample Preparation and Storage

- Mix diluted samples gently and thoroughly.
- Pipetting less than 2 µL is not recommended for optimal assay accuracy.
- Untreated samples should be stored at -20°C.
- Prepared samples can be stable for 1 week at +4°C.

8.1.1 **Tissue** (meat, chicken, duck, liver, shrimp, fish, egg, etc.)

8.1.1.1 Homogenize the sample at 10,000 rpm for 1 min.

8.1.1.2 Weigh 3 ± 0.05 g of the homogenized sample, place into centrifugal tube, add 3 mL **0.02 M PB Buffer**, mix gently and thoroughly.

8.1.1.3 Add 4 mL ethyl acetate and 2 mL Acetonitrile ($CH_3CN$), mix gently and thoroughly for 5 minutes.

8.1.1.4 Centrifuge at above 4,000 rpm at room temperature (20-25 °C) for 10 min.

8.1.1.5 Transfer 2 mL of supernatant (approx 1 g sample) into a new centrifugal tube, dry completely with a gentle stream of nitrogen or rotary evaporation at 56 °C.

8.1.1.6 Add 1 mL N-hexane, then add 1 mL of the diluted 1X Sample Prep, shake strongly for 30 seconds.

8.1.1.7 Centrifuge at 4,000 rpm at room temperature for 5 min, remove the upper layer.

8.1.1.8 Assay samples neat (do not dilute).

8.1.2 **Honey**

8.1.2.1 Weight 1.0±0.05 g honey into 50 mL centrifugal tube, then add 1 mL **0.5 M HCl**. Incubate at 37°C for 30 minutes.

8.1.2.2 Add 2.5 mL **0.2 M NaOH** (adjust pH to 5), then add 4 mL ethyl acetate

8.1.2.3 Shake for 5 min then centrifuge greater than 4,000 rpm at room temperature (20-25°C) for 10 minutes.

8.1.2.4 Transfer 2 mL supernatant into a new centrifugal tube, dry completely with nitrogen by rotary evaporation at 56 °C.

8.1.2.5 Add 0.5 mL of the **1X Sample Prep Buffer** and dissolce for 30 seconds.

8.1.2.6 Assay samples neat (do not dilute).

## 9. Assay Procedure

• Equilibrate all reagents and materials to ambient room temperature prior to use in the procedure.

• Temperature regulation for all procedures and incubations to 25°C is recommended for optimal reproducibility.

**9.1** Determine the required number of wells and return any remaining unused wells and desiccant to the pouch.

**9.2** Add 50 µL of standards or samples into wells of the **Tylosin Microplate**. At least two replicates of each standard, sample or blank is recommended.

**9.3** Immediately add 50 µL of the **Sulfamethoxine Rabbit Detector Antibody** and 50 µL of the **Anti-Rabbit / HRP Conjugate** to each well.

**9.4** Cover the plate with the well plate lid, gently mix and incubate for 40 minutes.

**9.5** Remove the plate lid and discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle or aspiration.

**9.6** Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

**9.7** Wash plate 5 times with **1X Wash Buffer** as follows:

9.7.1 Add 250 µL of **1X Wash Buffer** to each assay well.

9.7.2 Incubate for 30 seconds.

9.7.3 Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle.

9.7.4 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

9.7.5 Repeat steps 10.7.1 through 10.7.4 **four** more times.

**9.8** Add 50 µL of prepared **Detection Reagent A** and 50 µL of **Detection Reagent B** to each well.

**9.9** Gently mix the plate and incubate for 15 minutes **in the dark**.

**9.10** Add 50 µL of **Stop Solution** to each well and mix gently. Well color should change to yellow immediately. Add the **Stop Solution** in the same well order as done for the **Detection Reagents**.

**9.11** Read the O.D. absorbance at 450 nm with a standard microplate reader within 5 minutes of stopping the reaction in step 10.10. If wavelength correction is available, set to 540 nm or 570 nm.

# 10. Calculation of Results

For analysis of the assay results, first derive the **Percent Relative Absorbance** for each sample or standard by calculating the mean absorbance between replicate wells. This value is then divided by the mean replicate Blank (0 ppb) absorbance, then multiplying by 100%:

$$\text{Percent Relative Absorbance (Sample or Standard)} = \frac{\text{Mean Absorbance (Sample or Standard)}}{\text{Mean Blank Absorbance}} \text{ X } 100\%$$

A standard curve is generated by plotting the **Percent Relative Absorbance** of each standard serial dilution point vs. the respective standard concentration. The Tylosin concentration contained in the samples can be interpolated by using linear regression of each sample **Percent Relative Absorbance** against the standard curve. This is best achieved using curve fitting software.

**Note:** If wavelength correction readings were available, subtract the readings at 540 nm or 570 nm from the readings at 450 nm. This may provide greater reading accuracy.

**Note:** If the samples measured were diluted, multiply the derived mean sample concentration by the dilution factor for a final sample concentration.

# 11. Typical Expected Data

## 11.1 Limit of Detection

Derived by linear regression of OD450 of the Mean Blank + 2xSD

| Sample | Mean Recovery |
|---|---|
| Tissue,Liver | 3 ppb |
| Honey | 1.5 ppb |

## 11.2 Reproducibility

Three samples of known concentrations were measured in 20 replicates within an assay plate to asses intra-assay reproducibility:

Mean Intra-Assay Precision - ≤8% (n = 3 x 20)

Three samples of known concentrations were measured across 40 replicate assays inter-assay reproducibility:

Mean Inter-Assay Precision - ≤12% (n= 3 x 40)

### 11.3 Cross-Reactivity Rate

| Substance | Cross Reactivity Rate |
|---|---|
| TYL | 100% |
| EM | <1% |
| Other macrolides | <1% |

### 11.4 Recovery

The recovery of Tylosin spiked at levels throughout the range of the assay in various matrices was evaluated. Samples were diluted prior to assay as directed in the Sample Preparation section.

| Sample | Mean Recovery |
|---|---|
| Tissue,Liver | 85% +/- 10% |
| Honey | 85% +/- 15% |

## 12. Technical Resources

**Technical Support:**

For optimal service please be prepared to supply the lot number of the kit used.

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