Tilmicosin ELISA Kit

02/21

(Catalog # E4943-100, 96 assays, Store at 4°C)

I. Introduction:

Tilmicosin is a semi-synthetic macrolide antibiotic that is structurally similar to Tylosin. As a macrolide, its main function is to bind to 23s rRNA of the 50S ribosomal subunit of the bacteria. In doing so, it prevents the transpeptidation/translocation step and thereby inhibits bacterial protein synthesis. It targets both gram-positive and gram-negative bacteria that cause respiratory infection in cattle and pigs. BioVision's Tilmicosin ELISA kit is used to quantitatively measure Tilmicosin in muscle and liver tissue, honey, milk, and egg samples. The kit is based on the Competitive ELISA principle. Samples and standards are added to the microwell plate that is pre-coated with an antigen and competes for binding to the anti-Tilmicosin antibody. The HRP conjugate is added to each well and any unattached conjugates are washed off using Wash Buffer. The HRP enzymatic reaction is detected by the addition of substrate reagents. Finally, the reaction is terminated with an acidic stop solution. The color developed is inversely proportional to the concentration of Tilmicosin in the samples.

II. Application:

This ELISA kit is used for *in vitro* quantitative determination of Tilmicosin
Detection Limit: 0.5ppb for muscle (Method 1), 10ppb for muscle (Method 2), 10ppb for liver, 1ppb for honey, 5ppb for milk and eggs
Sensitivity: 0.5ppb
Cross reaction: Tilmicosin 100%, Tylosin 1%

III. Sample Type:

Tissue (muscle, liver), Honey, Milk, Eggs

IV. Kit Contents:

Components	E4943-100	Part No.
Micro ELISA Plate	8 X 12 Strips	E4943-100-1
Standard (S0 – S5)	1 ml X 6	E4943-100-2
HRP Conjugate	5.5 ml	E4943-100-3
Antibody working solution	5.5 ml	E4943-100-4
Substrate A	6 ml	E4943-100-5
Substrate B	6 ml	E4943-100-6
Stop Solution	6 ml	E4943-100-7
Wash Buffer (20X)	40 ml	E4943-100-8
Redissolving Solution (2X)	50 ml	E4943-100-9
Plate Sealer	3	E4943-100-10

V. User Supplied Reagents and Equipment:

- Chemicals: deionized water, Methanol, Ethyl acetate, $NaHCO_3$, Na_2CO_3, n-Hexane
- Microplate reader, nitrogen evaporator
- Clean eppendorf tubes and graduated cylinders for preparing standards or sample dilutions
- Absorbent paper

VI. Storage and Handling:

The entire kit may be stored at 4°C for up to 12 months from the date of shipment. Opened kit may be stable for 1 month at 4°C.

VII. Reagent and Sample Preparation:

Note: Bring all reagents to room temperature (20-25°C) 30 minutes before use.
Before using the kit, spin tubes and bring down all components to the bottom of tubes.

1. **Wash Buffer (1X):** Dilute 15 mL of **Wash buffer (20X)** with deionized water to 300 ml

2. **Standards Preparation:** Ready-to-use standards are as follows

Standards	S0	S1	S2	S3	S4	S5
Conc. (ppb)	0	0.5	1.5	4.5	13.5	40.5

3. **Sample Preparation:**
Note: The prepared sample maybe stored for up to one day at 2-8°C.

Sample pre-treatment: The following method must be used for pre-treatment of any kind of sample:
Note: Only the disposable tips can be used for the experiments and the tips must be changed when used for absorbing different reagents.

Solution preparation before sample pre-treatment:

1) **0.1M CB buffer (pH 10.6):** Take 0.932 grams of **Na_2CO_3** and 0.1 grams of **$NaHCO_3$** and make up the volume to 100 ml with deionized water.
2) **0.5M CB buffer (pH 10.6):** Take 4.66 grams of **Na_2CO_3** and 0.5 grams of **$NaHCO_3$** and make up the volume to 100 ml with deionized water.
3) **Reconstitution buffer (1X):** Take 1 part of **Reconstitution buffer (2X)** and dissolve it in 1 part of deionized water.
4) **Sample diluent:** Take 19 parts of **Reconstitution buffer (1X)** and dissolve it in 1 part of **Methanol**.

Sample Preparation and pre-treatment (for muscle tissue – Method 1):

Detection limit: 0.5ppb

- Take 2 ± 0.05 grams of the homogenized tissue sample into a centrifuge tube. Add 2 ml of **0.1M CB buffer (pH 10.6)**, mix well, and then add 8 ml **Ethyl acetate**, mix for 3 mins, and centrifuge at 3000 r/min for 5 mins.
- Take 4 ml of the upper organic phase into a glass tube and blow-dry with nitrogen evaporator or water bath at 50 – 60 ^{0}C.
- After blow-dry, dissolve the dry residue in 1 ml **n-Hexane**, then add 1 ml of the **Reconstitution buffer (1X)**, mix for 1 min, centrifuge at above 3000 r/min for 5 mins.
- Remove the upper liquid. Take 50 µl of the lower liquid for the analysis.
- **Fold of dilution of the sample: 1**

Sample Preparation and pre-treatment (for muscle tissue – Method 2):

Detection limit: 10ppb

- Take 1 ± 0.05 grams of the homogenized tissue sample into a centrifuge tube. Add 4 ml of **Wash buffer (1X)**. Mix for 3 mins, and then centrifuge at 3000 r/min for 5 mins at room temperature (RT).
- Take 200 µl of the supernatant into a new centrifuge tube and add 600 µl of **Reconstitution buffer (1X)**. Mix for 30 secs.
- Take 50 µl for the analysis.
- **Fold of dilution of the sample: 20**

Sample Preparation and pre-treatment (for liver tissue):

Detection limit: 0.5ppb

- Take 1 ± 0.05 grams of the homogenized liver tissue sample into a centrifuge tube. Add 2 ml of **0.5M CB buffer (pH 10.6)**, mix well, and then add 8 ml **Ethyl acetate**, mix for 3 mins, and centrifuge at 3000 r/min for 5 mins.
- Take 2 ml of the upper organic phase into a glass tube and blow-dry with nitrogen evaporator or water bath at 50 – 60 ^{0}C.
- After blow-dry, dissolve the dry residue in 1 ml **n-Hexane**, then add 1 ml of the **Reconstitution buffer (1X)**, mix for 1 min, centrifuge at above 3000 r/min for 5 mins.
- Remove the upper liquid. Take 100 µl of the lower liquid and add 400 µl of the **Reconstitution buffer (1X)**. Mix for 30 secs.
- Take 50 µl for the analysis.
- **Fold of dilution of the sample: 20**

Sample Preparation and pre-treatment (for honey sample):

Detection limit: 1ppb

- Take 2 ± 0.05 grams of the honey sample into a centrifuge tube. Add 2 ml of **0.1M CB buffer (pH 10.6)**, mix well, and then add 8 ml **Ethyl acetate**, mix for 3 mins, and centrifuge at 3000 r/min for 5 mins.
- Take 2 ml of the upper organic phase into a glass tube and blow-dry with nitrogen evaporator or water bath at 50 – 60 ^{0}C.
- After blow-dry, add 1 ml of the **Reconstitution buffer (1X)** to the sample residue, and mix for 30 secs.
- Take 50 µl for the analysis.
- **Fold of dilution of the sample: 2**

Sample Preparation and pre-treatment (for egg sample):

Detection limit: 5ppb

- Take 100 µl of the homogenized egg sample and add 900 µl of the **Sample diluent**. Mix for 30 secs.
- Take 50 µl for the analysis.
- **Fold of dilution of the sample: 10**

Sample Preparation and pre-treatment (for milk sample):

Detection limit: 5ppb

- Take 100 µl of the fresh milk sample and add 900 µl of the **Sample diluent**. Mix for 30 secs.
- Take 50 µl for the analysis.
- **Fold of dilution of the sample: 10**

VIII. Assay Protocol:

Note: Bring all reagents and samples to room temperature 30 minutes prior to the assay.

FOR RESEARCH USE ONLY! Not to be used on humans.

It is recommended that all standards and samples be run at least in duplicate.
A standard curve must be run with each assay.

1. Add 50 µl of the **sample or standards** to separate duplicate wells. Add 50 µl of the **HRP Conjugate**, and then add 50 µl **Antibody working solution** into each well. Mix gently by shaking the plate manually, seal the microplate with the plate sealer, and incubate at 25 ^{0}C for 30 mins.
2. Remove the plate sealer carefully, aspirate liquid out of microwells, and add 300 µl of **Wash Buffer (1X)** to each well. Wash for 30 secs, and then discard the buffer. Repeat the washing step five times. After the final wash step, tap to dry (if there are the bubbles after tapping, remove them with the clean tips).
3. Add 50 µl of the **Substrate A** and then 50 µl of the **Substrate B** into each well. Mix gently for 5 secs by shaking the plate manually, and incubate at 25 ^{0}C for 15 mins at dark.
4. Add 50 µl of the **Stop Solution** into each well. Mix gently by shaking the plate manually. Set the wavelength of the microplate reader at 450 nm to determine the OD value (Recommend reading the OD value at the wavelength 450 nm within 5 mins).

IX. Calculation:

- **Quantitative determination**

The mean values of the absorbance values obtained for the standards and the samples are divided by the absorbance value of the first standard (zero standard) and multiplied by 100%. The zero standard is thus made equal to 100% and the absorbance values are quoted in percentages.

Absorbance Value (%) = B/B_0 X 100%

B: The average absorbance value of the sample or standard

B_0 : The average absorbance value of the 0 ppb standard

To draw a standard curve: Take the absorbency value of standards as y-axis, logarithmic of the concentration of the Tilmicosin standards solution (ppb) as x-axis. The Tilmicosin concentration of each sample (ppb), which can be read from the calibration curve, is multiplied by the corresponding dilution factor of each sample followed, and the actual concentration of sample is obtained.

X. Related Products:

Streptomycin ELISA Kit (E4272)
Gentamicin (serum/urine) ELISA Kit (K4315)
Kanamycin ELISA Kit (K4210)
Quinolone ELISA Kit (E4530)
Ractopamine ELISA Kit (E4565)