Erythromycin ELISA Kit

02/21

(Catalog # E4955-100, 96 assays, Store at 4°C)

I. Introduction:

Erythromycin is a broad-spectrum antibiotic that belongs to the class of macrolides that contains Azithromycin, Spiramycin, and others. This bacteriostatic drug is produced by *Saccharopolyspora erythraea* and is effective against both gram-positive and gram-negative bacteria. The mechanism of action is that erythromycin binds to 23S rRNA of the 50S ribosomal subunit. In doing so, it prevents the transpeptidation/translocation step and thereby inhibits bacterial protein synthesis. Antimicrobials such as Erythromycin are extensively used in food-producing animals to treat infectious diseases. According to International Food Standards, Maximum Residue Limits (MRLs) for erythromycin for tissues of food-producing species (such as chicken and turkey) is 100 µg/kg (ppb) and in eggs is 50 µg/kg (ppb). BioVision's Erythromycin ELISA kit is used to quantitatively measure Erythromycin in tissue and milk 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-Erythromycin 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 Erythromycin in the samples.

II. Application:

This ELISA kit is used for *in vitro* quantitative determination of Erythromycin
Detection Limit: 2ppb for raw milk and egg, 5ppb for urine, serum and muscle (livestock), 10ppb for muscle (fish and shrimp)
Sensitivity: 0.1ppb
Cross reaction: Erythromycin 100%, Spiramycin, Valnemulin, Lincomycin, Kanamycin, Tilmicosin, Doramectin, Ivermectin, Eprinomectin, Moxidectin, Abamectin, Tylosin all < 0.1%

III. Sample Type:

Raw milk, Egg, Urine, Serum, Tissue (muscle)

IV. Kit Contents:

Components	E4955-100	Part No.
Micro ELISA Plate	8 X 12 Strips	E4955-100-1
Standard (S0 – S5)	1 ml X 6	E4955-100-2
HRP Conjugate	12 ml	E4955-100-3
Antibody working solution	10 ml	E4955-100-4
Substrate A	6 ml	E4955-100-5
Substrate B	6 ml	E4955-100-6
Stop Solution	6 ml	E4955-100-7
Wash Buffer (20X)	25 ml	E4955-100-8
Tissue diluent	50 ml	E4955-100-9
Plate Sealer	3	E4955-100-10

V. User Supplied Reagents and Equipment:

- Chemicals: deionized water, Methanol, N, N-Dimethylformamide (DMF), NaCl, NaOH, Conc. HCl
- 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:** To prepare 1X Wash Buffer, dilute 1 part of Wash buffer (20X) with 19 parts of deionized water. Prepare quantity as needed.

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

Standards	S0	S1	S2	S3	S4	S5
Conc. (ppb)	0	0.1	0.3	0.9	2.7	8.1

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

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

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) **6% DMF solution:** Take 6 ml of **N, N-Dimethylformamide** and make up the volume to 100 ml with deionized water. Mix well.
2) **2% NaCl solution:** Weigh 2 grams of **NaCl** and make up the volume to 100 ml with deionized water. Mix well
3) **0.05M NaOH solution:** Weigh 1 gram of **NaOH** and dissolve it in 500 ml of deionized water. Mix well.
4) **0.1M HCl solution:** Take 0.86 ml of **Conc. HCl** and make up the volume to 100 ml with deionized water. Mix well.
5) **Sample diluent:** Dilute 1 part of **Wash buffer (20X)** with 99 parts of deionized water. Mix well.

Sample Preparation and pre-treatment (for livestock muscle tissue samples):

Detection limit: 5ppb

- Take 1 ± 0.05 grams of the homogenized tissue sample into a 50 ml centrifuge tube. Add 4 ml of **0.05M NaOH solution**. Mix for 3 min. Centrifuge at 4000 r/min for 5 mins.
- Take 100 µl of the supernatant in a new tube and add 375 µl of **Tissue diluent**, and then add 25 µl of **0.1M HCl solution**. Mix for 10 secs. Centrifuge at 4000 r/min for 1 min.
- Take 20 µl of the supernatant for the analysis.
- **Fold of dilution of the sample: 20**

Sample Preparation and pre-treatment (for fish and shrimp muscle tissue samples):

Detection limit: 10ppb

- Take 2 ± 0.05 grams of the homogenized tissue sample into a 50 ml centrifuge tube. Add 1.2 ml of deionized water, and then add 2.8 ml **Methanol**. Mix for 1 min. Centrifuge at 4000 r/min for 10 mins.
- Take 200 µl of the supernatant in a new tube and add 800 µl of **Sample diluent**. Mix for 30 secs.
- Take 20 µl of the supernatant for the analysis.
- **Fold of dilution of the sample: 15**

Sample Preparation and pre-treatment (for raw milk samples):

Detection limit: 2ppb

- Take 1 ml of the fresh raw milk in a centrifuge tube and add 100 µl of **0.1M HCl solution**. Mix for 1 min. Centrifuge at 4000 r/min for 10 mins.
- Discard the upper fat layer, take 100 µl of the middle layer in a new tube; add 900 µl of **Sample Diluent**. Mix for 10 secs.
- Take 20 µl of this mixture for the analysis.
- **Fold of dilution of the sample: 10**

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

Detection limit: 2ppb

- Take 50 µl of the homogenized egg sample into a centrifuge tube. Add 950 µl of **2% NaCl solution**. Mix for 10 secs.
- Take 20 µl of this mixture for the analysis.
- **Fold of dilution of the sample: 20**

Sample Preparation and pre-treatment (for swine urine and bovine serum samples):

Detection limit: 5ppb

- Mix the sample thoroughly before performing the assay.
- Take urine/serum sample in a centrifuge tube and centrifuge at 4000 r/min for 5 mins.
- Take 100 µl of the supernatant urine/serum in a new tube; add 900 µl of **6% DMF solution**. Mix for 5 mins.
- Take 20 µl of this mixture 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.
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 20 µl of the **sample or standards** to separate duplicate wells. Add 80 µl of the **Antibody working solution** into each well. Mix gently for 10 secs by shaking the plate manually, seal the microplate with the plate sealer, and incubate in dark at 25 ^{0}C for 30 minutes in the dark.
2. Remove the plate sealer carefully, aspirate liquid out of microwells, and add 260 µl of **Wash Buffer (1X)** to each well. Wash for 30 secs, and then discard the buffer. Repeat the washing step four times. After the final wash step, tap to dry (if there are the bubbles after tapping, remove them with the clean tips).
3. Add 100 µl of **HRP Conjugate** in each well and incubate at 25 ^{0}C for 30 minutes in the dark.

4. Repeat washing as mentioned in **step 2**.
5. Add 50 µl of the **Substrate A** and then add 50 µl of the **Substrate B** into each well. Mix gently for 15 secs by shaking the plate manually, and incubate at 25 ^{0}C for 15 mins in dark.
6. Add 50 µl of the **Stop Solution** into each well. Mix gently for 10 secs 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: Plot the absorbance value of standards as y-axis, logarithmic of the concentration of the Ribavirin standards solution (ppb) as x-axis. The Ribavirin 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:

Sulfamethazine ELISA Kit (E4778)
Norfloxacin ELISA Kit (E4776)
Chlortetracycline ELISA Kit (E4782)
Ampicillin ELISA Kit (E4350)
Salbutamol (SALB) ELISA Kit (K4209)

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