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Colistin ELISA Kit

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(Catalog # E4946-100, 96 assays, Store at 4°C)

I. Introduction:

Colistin is a polymyxin antibiotic that is used as the last line of treatment for infections caused by multi-drug resistant organisms such as *Pseudomonas*, *Klebsiella*, and *Escherichia* species. The drug has been demonstrated to cause nephrotoxicity and neurotoxicity; however, the toxicity is dose-dependent and can be reversed by discontinuation of the treatment. The antibiotic is also used as a food additive in the animal farming industry, especially pig, cow, and rabbit farms to promote growth and treat intestinal infections caused by multidrug-resistant organisms. The drug is also administered with feed, milk, and water. BioVision's Colistin ELISA kit is used to quantitatively measure Colistin in muscle tissue and urine 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-Colistin 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 Colistin in the samples.

II. Application:

This ELISA kit is used for *in vitro* quantitative determination of Colistin
Detection Limit: 20ppb for muscle tissue, 10ppb for urine
Sensitivity: 1ppb
Cross reaction: Colistin 100%, polymyxin B--165%

III. Sample Type:

Tissue, Urine

IV. Kit Contents:

Components	E4946-100	Part No.
Micro ELISA Plate	8 X 12 Strips	E4946-100-1
Standard (S0 – S5)	1 ml X 6	E4946-100-2
HRP Conjugate	12 ml	E4946-100-3
Antibody working solution	10 ml	E4946-100-4
Substrate A	6 ml	E4946-100-5
Substrate B	6 ml	E4946-100-6
Stop Solution	6 ml	E4946-100-7
Wash Buffer (20X)	25 ml	E4946-100-8
Sample solution (20X)	40 ml	E4946-100-9
Sample Diluent	50 ml	E4946-100-10
Plate Sealer	3	E4946-100-11

V. User Supplied Reagents and Equipment:

- Chemicals: deionized water, Methanol (anhydrous)
- Microplate reader capable of measuring absorbance at 450 nm
- 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 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	1	3	9	27	81

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

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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) **Sample solution (1X):** Dilute 1 part of **Sample Solution (20X)** with 19 parts of deionized water and 1 part of **Methanol (anhydrous)**. Mix the solution well.

Sample Preparation and pre-treatment (for Chicken, duck, pork muscle tissue samples):

Detection limit: 20 ppb

- Take 1 ± 0.05 grams of the homogenized tissue sample into a 50 ml centrifuge tube. Add 1 ml of **Sample solution (1X)**. Mix for 5 mins. Centrifuge at 4000 r/min for 5 mins at room temperature (RT).
- Take 200 µl of the supernatant in a new centrifuge tube and add 200 µl of **Sample Diluent**. Mix for 4 mins.
- Take 20 µl of the liquid for the analysis
- **Fold of dilution of the sample: 10**

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

Detection limit: 10 ppb

- Take 50 µl of the centrifuged clear urine sample; add 450 µl of **Sample Diluent**. Mix for 1 min.
- Take 20 µl of the liquid 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 15 mins.
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 **HRP Conjugate** to each well. Cover the plate with plate sealer. Mix for 10 secs, and then incubate in dark at 25 ^{0}C for 15 mins in dark.
4. Repeat washing as 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 5 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 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 Colistin standards solution (ppb) as x-axis. The Colistin 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:

Clenbuterol ELISA Kit (E4564)
Furazolidone ELISA Kit (K4231)
Sulfonamides residue ELISA Kit (K4207)
Fluoroquinolones ELISA Kit (K4205)
Cimaterol ELISA Kit (E4771)

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