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Spectinomycin ELISA Kit

02/21

(Catalog # E4959-100, 96 assays, Store at 4°C)

I. Introduction:

Spectinomycin is an aminocyclitol drug produced by the soil microorganism *Streptomyces spectabilis*. The drug is effective against gram-positive and gram-negative bacteria and is used to treat gonorrheal infections. Spectinomycin works by binding to the 30S ribosomal subunit and thus inhibiting bacterial protein synthesis. The drug is used in animals not bred for human consumption to treat bacterial respiratory and enteric infections. BioVision's Spectinomycin ELISA kit is used to quantitatively measure Spectinomycin 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-Spectinomycin 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 Spectinomycin in the samples.

II. Application:

This ELISA kit is used for *in vitro* quantitative determination of Spectinomycin
Detection Limit: 5ppb for raw milk, 10ppb for muscle
Sensitivity: 0.2ppb
Cross reaction: Spectinomycin 100%, Streptomycin, Neomycin, Kanamycin, Gentamicin, Dihydrostreptomycin all < 0.01%

III. Sample Type:

Raw milk, Tissue (muscle)

IV. Kit Contents:

Components	E4959-100	Part No.
Micro ELISA Plate	8 X 12 Strips	E4959-100-1
Standard (S0 – S5)	1 ml X 6	E4959-100-2
HRP Conjugate	7 ml	E4959-100-3
Antibody working solution	7 ml	E4959-100-4
Substrate A	6 ml	E4959-100-5
Substrate B	6 ml	E4959-100-6
Stop Solution	6 ml	E4959-100-7
Wash Buffer (20X)	25 ml	E4959-100-8
Sample Solution (60X)	15 ml	E4959-100-9
Pork Solution (20X)	25 ml	E4959-100-10
Plate Sealer	3	E4959-100-11

V. User Supplied Reagents and Equipment:

- Chemicals: deionized water
- Microplate reader
- 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	0.2	0.6	1.8	5.4	16.2

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) **Sample solution (1X):** Dilute 1 part of **Sample solution (60X)** with 59 parts of deionized water. Mix well.
2) **Pork solution (1X):** Dilute 1 part of **Pork solution (20X)** with 19 parts of deionized water. Mix well.

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

Detection limit: 5ppb

- Bring raw milk to room temperature (RT). Gently swirl for 20 times to mix it thoroughly. **Note: Do not shake the milk vigorously or vortex. Precipitation of the milk may affect assay results.**
- Take 50 µl of the sample and add it to 750 µl of **Sample solution (1X)**. Mix for 10 – 20 secs at a low speed.
- Take 50 µl of the supernatant for the analysis.
- **Fold of dilution of the sample: 16**

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

Detection limit: 10ppb

- Take 1 ± 0.01 grams of the homogenized tissue sample into a 50 ml centrifuge tube. Add 5 ml of **Sample solution (1X).** Mix for 1 min. Centrifuge at 4000 r/min for 10 mins.
- Take 0.2 ml of the supernatant in a new centrifuge tube and add 0.4 ml of **Sample solution (1X)**. Mix for 20 secs.
- Take 50 µl of the supernatant for the analysis.
- **Fold of dilution of the sample: 18**

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

Detection limit: 10ppb

- Take 1 ± 0.01 grams of the homogenized tissue sample into a 50 ml centrifuge tube. Add 5 ml of **Sample solution (1X).** Mix for 10 secs. Centrifuge at 4000 r/min for 10 mins.
- Take 0.2 ml of the supernatant in a new centrifuge tube and add 0.4 ml of **Pork solution (1X)**. Mix for 20 secs.
- Take 50 µl of the supernatant for the analysis.
- **Fold of dilution of the sample: 18**

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 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 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 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 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 10 mins in dark.
4. 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)