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Oxytetracycline ELISA Kit

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(Catalog # E4957-100, 96 assays, Store at 4°C)

I. Introduction:

Oxytetracycline is a broad-spectrum antibiotic that belongs to the class of tetracyclines. It is effective against bacterial, chlamydial, and mycoplasma infections. The mechanism of action of this drug is to bind to 30S ribosomal subunit and prevent initiation of translation by binding of aminoacyl tRNA to the A site of the ribosome, thereby inhibiting protein synthesis. The drug can be used as a feed additive for food-producing animals to treat bacterial infections. It can also be used to promote growth in food-producing animals, promote milk and egg production and improve the efficiency of feed. BioVision's Oxytetracycline ELISA kit is used to quantitatively measure Oxytetracycline in tissue, milk, eggs, and feed 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-Oxytetracycline 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 Oxytetracycline in the samples.

II. Application:

This ELISA kit is used for *in vitro* quantitative determination of Oxytetracycline
Detection Limit: 6ppb for muscle, milk, egg, 400ppb for feed
Sensitivity: 0.3ppb
Cross reaction: Oxytetracycline 100%, Chlortetracycline 300%, Tetracycline 206%, Doxycycline 18.5%

III. Sample Type:

Tissue (muscle), Milk, Egg, Feed

IV. Kit Contents:

Components	E4957-100	Part No.
Micro ELISA Plate	8 X 12 Strips	E4957-100-1
Standard (S0 – S5)	1 ml X 6	E4957-100-2
HRP Conjugate	7 ml	E4957-100-3
Antibody working solution	7 ml	E4957-100-4
Substrate A	6 ml	E4957-100-5
Substrate B	6 ml	E4957-100-6
Stop Solution	6 ml	E4957-100-7
Wash Buffer (20X)	25 ml	E4957-100-8
Sample Diluent (20X)	50 ml	E4957-100-9
Plate Sealer	3	E4957-100-10

V. User Supplied Reagents and Equipment:

- Chemicals: deionized water, Trichloroacetic acid, $Na_2Fe(CN)_5NO.2H_2O$, $ZnSO_4.7H_2O$
- 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 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	0.3	0.9	2.7	8.1	24.3

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) **0.36M $Na_2Fe(CN)_5NO.2H_2O$ solution:** Weigh 10.7 grams of **$Na_2Fe(CN)_5NO.2H_2O$** and dissolve it in 100 ml of deionized water. Mix well. **Note: Prepare fresh solution before the experiment**.
2) **1M $ZnSO_4$ solution:** Weigh 28.8 grams of **$ZnSO_4.7H_2O$** and dissolve it in 100 ml of deionized water. Mix well.
3) **1% Trichloroacetic acid solution:** Weigh 1 gram of **Trichloroacetic acid** and dissolve it in 100 ml of deionized water. Mix well.
4) **Sample Diluent (1X):** Dilute 1 part of **Sample Diluent (20X)** with 19 parts of deionized water. Mix well.

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

Detection limit: 6ppb

- Take 1 ± 0.01 grams of the homogenized tissue sample into a 50 ml centrifuge tube. Add 9 ml of **Sample Diluent (1X).** Mix for 10 mins. Centrifuge at 4000 r/min at room temperature (RT) for 10 mins.
- Take 50 µl of the supernatant for the analysis.
- **Fold of dilution of the sample: 10**

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

Detection limit: 6ppb

- Take 3 ml of the fresh raw milk sample into a 5 ml centrifuge tube. Centrifuge at 4000 r/min at 4 – 10 ^{0}C for 10 mins. **If the centrifuge does not have a temperature regulator/refrigeration, chill the sample to approx. 10 ^{0}C before centrifugation**.
- Discard the upper fat layer, take 2 ml of the lower layer of milk in a new centrifuge tube and add 50 µl of **0.36M $Na_2Fe(CN)_5NO.2H_2O$ solution**. Mix for 90 secs. Add 50 µl of **1M $ZnSO_4$ solution**. Mix for 1 min.
- Centrifuge at 3000 r/min at RT for 10 mins. Take 50 µl of the supernatant in a new 2 ml centrifuge tube and add 450 µl of deionized water. Mix for 30 secs.
- Take 50 µl of the supernatant for the analysis.
- **Fold of dilution of the sample: 10**

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

Detection limit: 6ppb

- Take 1 ± 0.01 grams of the homogenized egg sample into a 50 ml centrifuge tube. Add 5 ml of deionized water**.** Mix for 2 mins. Centrifuge at 4000 r/min at room temperature (RT) for 10 mins.
- Take 1 ml of the supernatant in a new centrifuge tube and add 1 ml of **Sample Diluent (1X)**. Mix for 30 secs. Centrifuge at 4000 r/min at room temperature (RT) for 5 mins
- Take 50 µl of the supernatant for the analysis.
- **Fold of dilution of the sample: 12**

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

Detection limit: 400ppb

- Take 1 ± 0.01 grams of the feed sample into a 50 ml centrifuge tube. Add 5 ml of the **1% Trichloroacetic acid solution.** Mix for 10 mins. Centrifuge at 4000 r/min at room temperature (RT) for 10 mins.
- Take 40 µl of the supernatant in a new centrifuge tube and add 1.56 ml of **Sample Diluent (1X)**. Mix for 30 secs.
- Take 50 µl of the supernatant for the analysis.
- **Fold of dilution of the sample: 200**

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 **HRP Conjugate** and 50 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 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 15 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)