



Oxytetracycline ELISA Kit

Catalog Number KA6384

96 Assays

Version: 01

Intended for research use only

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Introduction

Product Description

This ELISA kit is used for in vitro quantitative determination of Oxytetracycline.

Background

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.

Principle of the Assay

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.

General Information

Materials Supplied

List of component

Components	Amount
Micro ELISA Plate	8 x 12 strips
Standard (S0–S5)	6 X 1 mL
HRP Conjugate	7 mL
Antibody working solution	7 mL
Substrate A	6 mL
Substrate B	6 mL
Stop Solution	6 mL
Wash Buffer (20X)	25 mL
Sample Diluent (20X)	50 mL
Plate Sealer	3

Storage Instruction

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.

Materials Required but Not Supplied

- ✓ Chemicals: deionized water, Trichloroacetic acid, $\text{Na}_2 \text{Fe}(\text{CN})_5 \text{NO} \cdot 2\text{H}_2\text{O}$, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$
- ✓ Microplate reader, Nitrogen evaporator
- ✓ Clean eppendorf tubes and graduated cylinders for preparing standards or sample dilutions
- ✓ Absorbent paper

Precautions for Use

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

Assay Protocol

Reagent Preparation

Note: Bring all reagents to room temperature (20-25°C) 30 minutes before use.

Before using the kit, spin the tubes and bring down all the components to the bottom of the tubes.

- ✓ Wash Buffer (1X): Dilute 1 part of Wash buffer (20X) with 19 parts of deionized water. Prepare quantity as needed.
- ✓ 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

Sample Preparation

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

- ✓ Sample Types: Tissue (muscle), Milk, Egg, Feed.
- ✓ 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 Na₂Fe(CN)₅NO.2H₂O solution: Weigh 10.7 grams of Na₂Fe(CN)₅NO.2H₂O and dissolve it in 100 mL of deionized water. Mix well. Note: Prepare fresh solution before the experiment.
 2. 1M ZnSO₄ solution: Weigh 28.8 grams of ZnSO₄.7H₂O 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: 6 ppb

 - 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: 6 ppb

 - Take 3 mL of the fresh raw milk sample into a 5 mL centrifuge tube. Centrifuge at 4000 r/min at 4–

10°C for 10 mins. If the centrifuge does not have a temperature regulator/refrigeration, chill the sample to approx. 10°C before centrifugation. deionized.

- 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 $\text{Na}_2\text{Fe}(\text{CN})_5\text{NO} \cdot 2\text{H}_2\text{O}$ 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: 6 ppb

- 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 this mixture for the analysis.
- Fold of dilution of the sample: 12

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

Detection limit: 400 ppb

- 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 this mixture for the analysis.
- Fold of dilution of the sample: 200

Assay Procedure

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°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 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°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).

Data Analysis

Calculation of Results

- ✓ 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.

$$\text{Absorbance Value (\%)} = B/B_0 \times 100\%$$

B: The average absorbance value of the sample or standard.

B₀: 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 Oxytetracycline standards solution (ppb) as x-axis. The Oxytetracycline 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.

Performance Characteristics

- ✓ Sensitivity: 0.3 ppb.
- ✓ Detection Limit: 6 ppb for muscle, milk, egg, 400 ppb for feed.
- ✓ Cross reaction: Oxytetracycline 100%, Chlortetracycline 300%, Tetracycline 206%, Doxycycline 18.5%.

Resources

Plate Layout

12								
11								
10								
9								
8								
7								
6								
5								
4								
3								
2								
1								
	A	B	C	D	E	F	G	H