Gentaur Europe BVBA Voortstraat 49, 1910 Kampenhout BELGIUM
Tel 0032 16 58 90 45 info@gentaur.com

Chlortetracycline ELISA Kit

11/19

(Catalog # E4782-100; 96 assays, Storage at 4°C)

I. Introduction:

Chlortetracycline is an antibiotic produced by some strains of Streptomyces aureofaciens. It inhibits protein synthesis (elongation) by preventing binding of aminoacyl-tRNA to the 30S subunit. It is effective against Gram-positive and to a lesser degree Gram-negative bacteria than tetracycline. BioVision Chlortetracycline ELISA Kit is based on Competitive ELISA principle. The micro-plate provided in this kit has been pre-coated with Chlortetracycline. During the reaction, Chlortetracycline in the samples or standard competes with Chlortetracycline coated on the plate for binding to the anti-Chlortetracycline antibody. Then Horseradish Peroxidase (HRP) conjugate is added to each micro plate well, and TMB substrate is for color development. There is a negative correlation between the OD value of samples and the concentration of Chlortetracycline. The concentration of Chlortetracycline in the samples can be calculated by comparing the OD of the samples to the standard curve.

II. Applications:

In vitro, quantitative determination of Chlortetracycline
Sensitivity: 0.1 ppb
Detection Range: Tissue, Liver, Eggs - 0.8 ppb, Honey - 4 ppb, Urine -1 ppb

Sample recovery rate: Tissue, Liver, Eggs -90%±20%; Honey -75%±20%; Urine -80±20%

Cross-reactivity: Chlortetracycline - 100%, Tetracycline -29%, Oxytetracycline -15%, Doxycycline -2.5%

III. Sample Type:

Tissue, Liver, Eggs, Honey, Urine

IV. Kit Contents:

Components	E4782-100	Part Number
Micro ELISA Plate	96 wells	E4782-100-1
Standard (1.0 ppm)	1 ml	E4782-100-2
HRP Conjugate	11 ml	E4782-100-3
Antibody Working Solution	5.5 ml	E4782-100-4
Substrate Reagent A	6 ml	E4782-100-5
Substrate Reagent B	6 ml	E4782-100-6
Stop Solution	6 ml	E4782-100-7
Wash Buffer (20X)	40 ml	E4782-100-8
Reconstitution Buffer (5X)	50 ml	E4782-100-9
Plate Sealer	3	E4782-100-10

V. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm
- Methanol, Trichloroacetic acid
- Clean Eppendorf tubes for preparing standards or sample dilutions

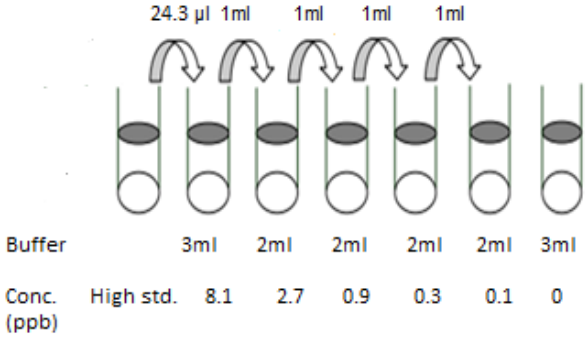
VI. Storage and Handling:

Store at 4ºC**.**

VII. Reagent and Sample Preparation:

Bring all reagents to room temperature before use. Before using the kit, spin tubes and bring down all components to the bottom of tubes.

- **Wash Buffer (20X):** Dilute 20X Concentrated Wash Buffer to 1x with deionized water.
- **Reconstitution Buffer (5X):** Dilute 5X Reconstitution Buffer with deionized water. Mix 5x Reconstitution Buffer (V): Deionized water (V) =1:4). The Reconstitution buffer can be store at 4℃ for a month.
- **1% Trichloroacetic acid Solution:** Dissolve 1 g of Trichloroacetic acid to 100 ml with deionized water
- **Standard:** Add 3 ml of Reconstitution Buffer into 0 ppb tube and 8.1 ppb. Add 2 ml of Reconstitution Buffer into 0.1 ppb tube, 0.3 ppb tube, 0.9 ppb tube and 2.7 ppb tube respectively.



- **Standard 6 (8.1 ppb):** Add 24.3 µl of 1.0 ppm high concentration standard into 8.1 ppb tube, mix well.
- **Standard 5 (2.7 ppb):** Add 1 ml of Standard 6 into 2.7 ppb tube, mix well.
- **Standard 4 (0.9 ppb):** Add 1 ml of Standard 5 into 0.9 ppb tube, mix well.
- **Standard 3 (0.3 ppb):** Add 1 ml of Standard 4 into 0.3 ppb tube, mix well.
- **Standard 2 (0.1 ppb):** Add 1 ml of Standard 3 into 0.1 ppb tube, mix well.
- **Standard 1 (0 ppb):** Reconstitution Buffer is as Standard Solution 1.

VIII. Sample Preparation:

Sample pretreatment:

- **<u>Pretreatment of tissue (livestock, shrimp, fish) liver, eggs sample:</u>**
  Weigh 2 ± 0.05 g of homogenate samples into centrifuge tube. Then add 4 ml of 1% Trichloroacetic acid Solution to centrifuge tube. Shake well for 2 min, centrifuge at 4000 r/min for 10 min at room temperature. Take 250 µl of the supernatant to another tube, and then add 750 µl of Reconstitution Buffer to dissolve it. Take 50 µl for analysis.
  Note: Sample dilution factor: 8, minimum detection limit: 0.8 ppb.

- **<u>Pretreatment of honey sample:</u>**
  Weigh 1±0.05 g of honey samples into a centrifuge tube. Then add 2 mL of 1% Trichloroacetic acid Solution. Mix well for 2 min, centrifuge at 4000 r/min for 10 min at room temperature. Take 100 µl of the supernatant to another tube. Add 1900 µl of Reconstitution Buffer. Mix well for 30s. Take 50 µl for analysis
  Note: Sample dilution factor: 40, minimum detection limit: 4 ppb.

- **Pretreatment of urine (swine) sample:**
  Take urine samples centrifuge at 4000 r/min for 10 min at room temperature. Dilute clear urine samples with Reconstitution Buffer for 10 times. (Urine: Reconstitution Buffer (Solution 2) (V) = 1:9). Take 50 µl for analysis.
  Note: Sample dilution factor: 10, minimum detection limit: 1 ppb

IX. Assay Protocol:

<u>Note:</u> 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 each **standard or samples** into appropriate wells.
2. Add 50 µl of Antibody **Working Solution**. Cover the plate with the sealer provided in the kit. Gently mix and incubate for 30 min. at 37°C.
3. Aspirate the solution from each well add 300 µl of **1x wash buffer** to each well. Leave it for 30 sec, aspirate the solution from each well and pat it dry against clean absorbent paper. Repeat this wash step 5 times.
4. Add 100 µl of **HRP Conjugate** to each well, incubate at 37°C for 30 min in dark.
5. Repeats wash Step 3.
6. Add 50 µl of **Substrate Reagent A** to each well and then add 50 µl of **Substrate Reagent B.** Cover with a plate sealer. Incubate for about 15 min at 37°C. Protect the plate from light.

   Note: the reaction time can be shortened or extended according to the actual color change, but not more than 30 min.
7. Add 50 µl of **Stop Solution** to each well. Note: adding the stop solution should be done in the same order as the substrate solution.
8. Read the absorbance in micro plate reader set to 450 nm reference wavelength 630 nm. This step should be performed within 10 min after stop reaction.

X. Calculation:

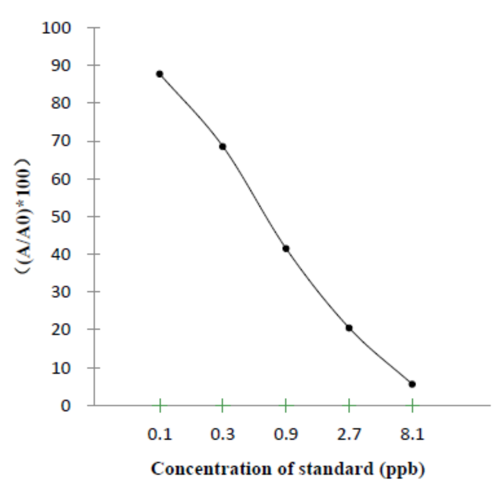
Create a standard curve by plotting the absorbance percentage of each standard on the y-axis against the log concentration on the x-axis to draw a semi logarithmic plot. Add average absorbance value of sample to standard curve to get corresponding concentration. If samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor.

Absorbance (%)=A/A_0 ×100%

A: Average absorbance of standard or sample

A_0 : Average absorbance of 0 ppb Standard

Typical standard curve and data is provided below for reference only. A standard curve must be run with each assay



Concentration of standard (ppb)	OD-1	OD-2	Average OD
0	1.9579	1.9853	1.9716
0.1	1.7434	1.7148	1.7291
0.3	1.3048	1.3965	1.3507
0.9	0.8342	0.8020	0.8181
2.7	0.4018	0.4046	0.4032
8.1	0.1082	0.1102	0.1092

XI. RELATED PRODUCTS:

- Tylosin ELISA Kit (E4779)
- Norfloxacin ELISA Kit (E4776)
- Diazepam ELISA Kit (E4772)
  Olaquindox ELISA Kit (E4781)

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