



## **Tetracycline (TET) ELISA Kit**

**Catalog Number. CSB-E12090f**

**This immunoassay kit allows for the in vitro quantitative determination of Tetracycline concentrations in honey, tissue(chicken, pork, duck), fish, shrimp, egg.**

This package insert must be read in its entirety before using this product.

### **If You Have Problems**

#### **Technical Service Contact information**

Phone: 86-27-87582341

Fax: 86-27-87196150

Email: [tech@cusabio.com](mailto:tech@cusabio.com)

Web: [www.cusabio.com](http://www.cusabio.com)

In order to obtain higher efficiency service, please ready to supply the lot number of the kit to us (found on the outside of the box).

### **PRINCIPLE OF THE ASSAY**

This assay employs the competitive enzyme immunoassay technique. The coupling antigen is pre-coated on the micro-well stripes. The Tetracycline in the testing sample competes with the coupling antigens pre-coated on the micro-well stripes for the antibodies against Tetracycline. After the addition of the enzyme conjugate, the TMB substrate is added for coloration. The optical density (OD) value of the testing sample has a negative correlation with the content of Tetracycline in it. This value is compared to the standard curve and the content of the corresponding Tetracycline is subsequently obtained.

### **DETECTION RANGE**

0.05ppb-40.5 ppb.

### **SENSITIVITY**

The minimum detectable dose of the kit is typically less than 0.05 ppb.

The sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest concentration that could be differentiated from zero. It was determined the mean O.D value of 20 replicates of the zero standard added by their three standard deviations.

### **Cross-reaction rate**

Tetracyclines	100%
Minocycline	125%
RoliTetracyclines	110%
Aureomycin	100%
Demethylchlor Tetracyclines	35%
Terramycin	58%
Doxycycline	45%

**Recovery rate**

chicken, duck, pork, fish, shrimp	85% ±10%
milk, honey	70% ±10%

**Detection limit**

A: Chicken, duck, pork (meat or liver), shrimp, fish, milk

Tetracyclines	1ppb
Minocycline	1ppb
RoliTetracyclines	1ppb
Aureomycin	1ppb
Demethylchlor Tetracyclines	3ppb
Terramycin	2ppb
Doxycycline	3ppb

B: Honey

Tetracyclines	2ppb
Minocycline	2ppb
RoliTetracyclines	2ppb
Aureomycin	2ppb
Demethylchlor Tetracyclines	6ppb
Terramycin	4ppb
Doxycycline	6ppb

## **PRECISION**

### **Intra-assay Precision (Precision within an assay): CV%<8%**

Three samples of known concentration were tested twenty times on one plate to assess.

### **Inter-assay Precision (Precision between assays): CV%<10%**

Three samples of known concentration were tested in twenty assays to assess.

## **LIMITATIONS OF THE PROCEDURE**

- **FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, dilute the samples and repeat the assay.
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Immunoassay, the possibility of interference cannot be excluded.

**MATERIALS PROVIDED**

Reagent	Quantity
Assay plate (96 tests)	1
Standard	6 x 1 ml
HRP-conjugate	1x 12 ml
Antibody	1 x 7 ml
Substrate A	1x 7 ml
Substrate B	1x 7 ml
Stop Solution	1x 7 ml
Wash Buffer	2 x 20ml (20xconcentrate)
Redissolving Solution	3 x 15 ml (5xconcentrate)

**STANDARD CONCENTRATION**

Standard	S0	S1	S2	S3	S4	S5
Concentration (ppb)	0	0.5	1.5	4.5	13.5	40.5

**STORAGE**

Store at 2 - 8°C. Do not use past kit expiration date.

**OTHER SUPPLIES REQUIRED**

- Microplate reader capable of measuring absorbance at 450 nm.
- An incubator which can provide stable incubation conditions up to 37°C±0.5°C.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- Absorbent paper for blotting the microtiter plate.
- 100 ml and 500 ml graduated cylinders.
- Deionized or distilled water.

- Pipettes and pipette tips.
- Test tubes for dilution.
- C<sub>18</sub> Column

### **PRECAUTIONS**

The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

#### **Note:**

- **Kindly use graduated containers to prepare the reagent.**
- Bring all reagents to room temperature (18-25°C) before use for 30min.
- Distilled water is recommended to be used to make the preparation for reagents or samples. Contaminated water or container for reagent preparation will influence the detection result.

### **REAGENT PREPARATION**

1. Wash Buffer: If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 ml of 20x concentrate Wash Buffer with deionized or distilled water to prepare 400 ml of Wash Buffer.
2. Redissolving Solution: The 5 concentrated Redissolving Solution is diluted with deionized water at 1:4 (eg:1 mL concentrated Redissolving Solution + 4 mL deionized water).
3. 3% Trichloroacetic acid: dissolve 3 g Trichloroacetic acid in deionized water to 100ml.
4. 1% Trichloroacetic acid: dissolve 1 g Trichloroacetic acid in deionized water to 100ml.
5. 0.1M NaOH Solution: dissolve 0.4 g NaOH in deionized water to 100 ml.
6. 0.1MNa<sub>2</sub>EDTA-Mcllvaine Buffer (pH4.0): dissolve 27.6g Na<sub>2</sub>HPO<sub>4</sub>•12H<sub>2</sub>O, 12.9 g C<sub>6</sub>H<sub>8</sub>O<sub>7</sub>•H<sub>2</sub>O and 37.2 g EDTA•Na<sub>2</sub>•2H<sub>2</sub>O in deionized water to 1000 ml.
7. CH<sub>3</sub>OH -0.02M H<sub>2</sub>C<sub>2</sub>O<sub>4</sub> Solution: dissolve 1.8 g H<sub>2</sub>C<sub>2</sub>O<sub>4</sub> in CH<sub>3</sub>OH to 1L

**Note:**

1. CUSABIO is only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.
2. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
3. Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
4. Tissue or cell extraction samples prepared by chemical lysis buffer may cause unexpected ELISA results due to the impacts of certain chemicals.
5. Owing to the possibility of mismatching between antigen from other resource and antibody used in our kits (e.g., antibody targets conformational epitope rather than linear epitope), some native or recombinant proteins from other manufacturers may not be recognized by our products.

## **SAMPLE COLLECTION AND STORAGE**

A: Tissues(pork, chicken, duck, porcine liver, shrimp, fish, egg)

- (1) Homogenize the sample at 10000 r/min for 1 min.
- (2) Weigh  $2.0 \pm 0.05$  g of the homogenized sample, put into 50ml centrifugal tube, add 4ml 3% Trichloroacetic acid, shake properly for 5min. Centrifuge at more than 4000 r/min for 10 min at room temperature.
- (3) Transfer 100 $\mu$ L supernatant into a new centrifugal tube, add 100 $\mu$ L 0.1M NaOH Solution and 800 $\mu$ L diluted Redissolving Solution. Shake for 30s. (If it's not clear, centrifuge at more than 4000 r/min for 10 min at room temperature.)
- (4) Take 50 $\mu$ L for further analysis. Fold of dilution of the samples: 20

B: Honey

- (1) Weight  $1 \pm 0.05$  g honey into 50 mL centrifugal tube, then add 2 mL 1% Trichloroacetic acid, shake for 5 min. Centrifuge at more than 4000 r/min for 10 min at room temperature.
- (2) Take 100 $\mu$ L supernatant into a new centrifugal tube, add 1900 $\mu$ L diluted Redissolving Solution. Shake for 30s.
- (3) Take 50 $\mu$ L for further analysis. Fold of dilution of the sample: 40

C: Tissues(pork, chicken, duck, porcine liver, shrimp, fish) by C<sub>18</sub> column

- (1) Homogenize the sample at 10000 r/min for 1 min.
- (2) Weigh  $5 \pm 0.05$  g of the homogenized sample, put into 50ml centrifugal tube, add 25ml 0.1MNa<sub>2</sub>EDTA-Mclvaine Buffer(pH4.0), shake properly for 30min. Centrifuge at more than 4000 r/min for 10 min at room temperature.
- (3)

- (4) Transfer all supernatant into a new centrifugal tube, extract the precipitate with 25ml 0.1MNa<sub>2</sub>EDTA-McIlvaine Buffer(pH4.0) again.
- (5) Put the two supernatant together, and filtered by filter paper. Take 5mL filter solution for purification.

**Purification procedure for 5mL filter solution**

- (1) Take 3 mL CH<sub>3</sub>OH(100%) to wash the C<sub>18</sub> column.
- (2) Take 2 mL deionized water to wash the C<sub>18</sub> column.
- (3) Add the 5 mL filter solution to the C<sub>18</sub> column, 15 drop/min.
- (4) Wash the column by 3 mL deionized water.
- (5) Empty the water in the column.
- (6) Washed by 1 mL CH<sub>3</sub>OH -0.02M H<sub>2</sub>C<sub>2</sub>O<sub>4</sub> Solution, 15 drop/min. gather the wash solution.
- (7) Diluted the wash solution with diluted Redissolving Solution at 1:9(50 μL sample + 450 μL diluted Redissolving Solution),mix for 30s.
- (8) Take 50 μL for analysis. Fold of dilution of the sample: 20

## ASSAY PROCEDURE

**Bring all reagents and samples to room temperature before use. Centrifuge the sample again after thawing before the assay. It is recommended that all samples and standards be assayed in duplicate.**

1. Prepare all reagents and samples as directed in the previous sections.
2. Determine the number of wells to be used and put any remaining wells and the desiccant back into the pouch and seal the ziploc, store unused wells at 4°C.
3. Dilute 50 µL of Standards (S1-S6) with 450 µL diluted Redissolving Solution, respectively.
4. Add 50µl of **Standard** or **Sample** per well. Standard and Samples need test in duplicate.
5. Add 50µl of **Antibody** to each well. Mix well and then incubate for 30 min at 37°C.
6. Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with **Wash Buffer** (250µl) using a squirt bottle, multi-channel pipette, manifold dispenser, or autowasher, and let it stand for 2 minutes, complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
7. Add 50µl of **HRP-conjugate** to each well. Mix well and then incubate for 30 min at 37°C.
8. Repeat the aspiration/wash process for five times as before.
9. Add 50µl of **Substrate A** and 50µl of **Substrate B** to each well, mix well. Incubate for 15 minutes at 37°C. Keeping the plate away from drafts and other temperature fluctuations in the dark.
10. Add 50µl of **Stop Solution** to each well, gently tap the plate to ensure thorough mixing.
11. Determine the optical density of each well within 10 minutes, using a microplate reader set to 450 nm.

Note:

1. The final experimental results will be closely related to validity of the products, operation skills of the end users and the experimental environments.
2. Samples or reagents addition: Please carefully add samples to wells and mix gently to avoid foaming. Do not touch the well wall as possible. For each step in the procedure, total dispensing time for addition of reagents or samples to the assay plate should not exceed 10 minutes. This will ensure equal elapsed time for each pipetting step, without interruption. Duplication of all standards and specimens, although not required, is recommended. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
3. Incubation: To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods between incubation steps. Once reagents have been added to the well strips, DO NOT let the strips DRY at any time during the assay. Incubation time and temperature must be observed.
4. Washing: The wash procedure is critical. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Solution by aspirating or decanting and remove any drop of water and fingerprint on the bottom of the plate. Insufficient washing will result in poor precision and falsely elevated absorbance reading. When using an automated plate washer, adding a 30 second soak period following the addition of wash buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
5. Controlling of reaction time: Observe the change of color after adding Substrates (e.g. observation once every 10 minutes). Substrates should change from colorless or light blue to gradations of blue. If the color is too deep, add Stop Solution in advance to avoid excessively strong reaction which will result in inaccurate absorbance reading.
6. Substrates are easily contaminated. Substrates should remain colorless or light blue until added to the plate. Please protect it from light.
7. Stop Solution should be added to the plate in the same order as the Substrates. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrates.

## CALCULATION OF RESULTS

Using the professional soft "Curve Exert 1.3" to make a standard curve is recommended, which can be downloaded from our web.

- (1) 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.

$$\text{Absorbency value (\%)} = \frac{B}{B_0} \times 100\%$$

B —absorbance standard (or sample)

B<sub>0</sub> —absorbance zero standard

- (2) To draw a standard curve: Take the absorbance value of standards as y-axis, semi logarithmic of the concentration of the BP standards solution (ppb) as x-axis.

The BP 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.