TECHNICAL MANUAL

TCs (Tetracyclines) ELISA Kit

- **SKU CODE:** FSES0029
- **SIZE:** 96T
- **DETECTION PRINCIPLE:** Competitive
- **RUO:** Research-Use-Only

Revised: 04/2026

TCs (Tetracyclines) ELISA Kit

Please read entire manual carefully before starting experiment. DO NOT mix reagents and use reagents from different kits or batches to prevent assay failure.

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1. Key Features

Detection Method:

Competitive (Antigen Coated)

Sample Type:

Muscle, Liver, Eggs, Milk, Milk powder, Honey & Urine

Detection limit:

Muscle, Liver, Eggs, Honey, Milk---2 ppb; Milk powder---4 ppb; Urine---0.5 ppb

Cross-Reactivity:

Tetracycline---100%; Chlortetracycline---16.7%; Oxytetracycline---107%; Doxycycline---4.2%.

Sample recovery rate:

Muscle, Liver, Eggs, Milk, Milk powder---75%±20%

Honey, Urine---80%±20%

2. Storage & Expiry

Assay Genie ELISA Kits are shipped on ice packs. Please store this ELISA Kit and/or components at 2-8^{O}C. Date of expiration is on the ELISA Box label.

3. Product Description

The Assay Genie Tetracyclines (TCS) ELISA Kit is a highly sensitive assay for the quantitative measurement of a Tetracyclines in samples, such as muscle, honey and more.

This kit is based on the principle of a Competitive enzyme-linked immunosorbent assay (ELISA) principle, where the microtiter wells are pre-coated with Tetracyclines coupled antigen. In this setup, the Tetracyclines present in the sample competes with the immobilized antigen for binding to a limited amount detection antibody.

When the sample and the detection antibody are added simultaneously, higher concentrations of Tetracyclines in the sample will block more antibody binding to the coated antigen.

After washing away unbound materials, HRP-Streptavidin is added, which binds to the biotin-labelled antibodies attached to the well. The TMB substrate is then added, producing a color reaction. The color intensity is inversely proportional to the concentration of Tetracyclines in the sample, higher sample concentrations result in a weaker color signal (lower OD at 450 nm).

The concentration of Tetracyclines is determined by comparing sample OD values to the standard curve.

4. Kit Contents

No	Component Name	Specifications
1	ELISA Microtiter Plate	96 wells
2	High Concentrated Standard (1.0 ppm)	1 mL each (ppb=ng/mL=ng/g)
3	Standard Liquid (empty bottle)	(0 ppb, 0.05 ppb, 0.15 ppb, 0.45 ppb, 1.35 ppb, 4.05 ppb)
4	HRP Conjugate	1 mL
5	Antibody Working Solution	5.5 mL
6	Substrate Reagent A	6 ml
7	Substrate Reagent B	6 mL
8	Stop Solution	6 mL
9	20X Concentrated Wash Buffer	40 mL
10	5x Reconstitution Buffer	50 mL
12	Plate Sealer	1 pieces
12	Sealed Bag	1 piece
13	Manual	1 copy

Note: *Return any unused wells to their original foil bag and reseal them together with the desiccant provided. Store at 2-8°C.*

Additional materials required:

1. 37°C incubator.
2. Plate Reader with 450nm filter.
3. Precision pipettes and disposable pipette tips.
4. Distilled water.
5. Disposable tubes for sample dilution.
6. Absorbent paper.
7. Reagents: HCl and Methanol anhydrous

5. Precautions

1. This kit is ideal for research purposes only and not for diagnostics or therapeutic uses.

2. To identify the concentration of your target, a pilot experiment using standards and a small number of samples is recommended.

3. Store all components as listed in this manual. Do not use the ELISA Kit after its expiration date

4. Ensure unopened and unused plate is kept dry to avoid contamination.

5. Before using the kit, centrifuge tubes to spin down standard & antibodies.

6. Avoid light for storage of TMB reagents.

7. Wash steps are critical for the success of the assay, deviations from wash steps may cause false positives and result in a high background.

8. Duplicate wells are recommended for both standard and sample testing.

9. Do not let the microplate wells dry during assay.

10. Do not reuse tips and tubes to avoid cross contamination.

11. Avoid using the reagents from different batches together.

6. Sample Preparation

The procedures outlined in this document are provided as general recommendations for sample preparation in ELISA assays. Due to the variability of biological samples and specific assay requirements, users are advised to optimize protocols based on their own experimental conditions.

***Note**: For information regarding validation data in specific samples, please contact our Technical Support Team at techsupport@assaygenie.com.*

General Considerations

To prevent denaturation or degradation of target proteins, it is recommended to process samples promptly and store them under appropriate conditions.

- **Storage Conditions**:
  - **Short-term**: 2-8 °C for up to 5 days.
  - **Medium-term**: -20 °C for up to 6 months.
  - **Long-term**: -80 °C or cryopreservation in liquid nitrogen.
- **Thawing Protocol**: Frozen samples should be thawed rapidly in a 15-25 °C water bath to minimize ice crystal-induced damage. Thawed samples can be analyzed immediately or stored temporarily at 2-8 °C.
- **Freeze-Thaw Cycles**: Repeated freeze-thaw cycles should be strictly avoided due to their detrimental effect on protein stability.

Solution Preparation

Bring all reagents and samples to room temperature (25°C) prior to use. Prepare only the required volume of each solution based on the number of samples to be processed.

- **Solution 1 - Extraction Solution:** Dilute 4.3 mL of HCl to 50 mL with deionized water, and add 450 mL with Methanol anhydrous, mix fully.

- **Solution 2 - Reconstitution Buffer:** Dilute the 5×Reconstitution Buffer with deionized water. (5×Reconstitution Buffer (V): deionized water (V) =1:4). The Reconstitution buffer can be store at 4℃ for a month.
- **Solution 3 - Wash Buffer:** Dilute the 20×Concentrated Wash Buffer with deionized water. (20×Concentrated Wash Buffer (V): Deionized water (V) =1:19)

Sample Pretreatment Procedures

A. Muscle (livestock, fish, shrimp), liver, eggs sample:

- Remove fat from fresh sample (except eggs). Homogenize the representative sample with a homogenizer and mix thoroughly.
- Weigh 1±0.05 g of homogenate sample into centrifuge tube, add 2 mL of Extraction Solution (Solution 1). Vortex for 2 minutes, the sample is fully mixed with the liquid. Centrifuge at 4000 r/min for 5 minutes at room temperature.
- Take 50 µL of the supernatant to another centrifuge tube (Be careful not to absorb white float), add 950 µL of Reconstitution Buffer (Solution 2) and mix thoroughly.
- Take 50 µLfor analysis.

Note: Sample dilution factor: 40 **| Detection limit:** 2 ppb.

B. Honey sample

- (Weigh 1±0.05 g of fresh honey sample into the centrifuge tube, add 1 mL of Extraction Solution (Solution 1). Vortex for 2 minutes.
- Take 50 µLof the Step (1) liquid to another centrifuge tube, add 950 µL of Reconstitution Buffer (Solution 2) and mix thoroughly.
- Take 50 µLfor analysis.

Note: Sample dilution factor: 40 **| Detection limit:** 2 ppb.

C. Urine (swine) sample

- Take 0.5 mL of fresh sample, centrifuge at 4000 r/min at room temperature for 10 minutes.
- Take 20 µL of supernatant, add 180 µL of Reconstitution Buffer (Solution 2), and mix thoroughly.
- Take 50 µL for analysis.

Note: Sample dilution factor: 10 **| Detection limit:** 0.5 ppb.

D. Milk sample

- Take 1 mL of fresh milk sample, add 2 mL of Extraction Solution (Solution 1), vortex for 2 minutes. Centrifuge at 4000 r/min at room temperature for 5 minutes.
- Take 50 µL of supernatant, add 950 µL of Reconstitution Buffer (Solution 2), and mix thoroughly.
- Take 50 µL for analysis.

Note: Sample dilution factor: 40 **| Detection limit:** 2 ppb.

E. Milk powder sample

Weigh 1±0.05 g of fresh sample, add 4 mL of Extraction Solution (Solution 1), vortex for 2 minutes. Centrifuge at 4000 r/min at room temperature for 5 minutes. (2) Take 50 µL of supernatant, add 950 µL of Reconstitution Buffer (Solution 2), and mix thoroughly. (3) Take 50 µLfor analysis.

Note: Sample dilution factor: 80 | **Detection limit**: 4 ppb.

F. Other Sample Types

For more information about how to process other sample types, please contact our Tech Support Team at techsupport@assaygenie.com.

7. Reagent Preparation

Manual Washing

Discard the solution in the plate without touching the side of the wells. Clap the plate on absorbent filter paper or other absorbent material. Fill each well completely with 350 µl wash buffer and soak for 1 to 2 mins, then aspirate contents from the plate, and clap the plate on absorbent filter paper or other absorbent material. Repeat this procedure for the designated number of washes.

Automated Washing

Aspirate all wells, then wash plate with 350 µl wash buffer. After the final wash, invert plate, and clap the plate on absorbent filter paper or other absorbent material. It is recommended that the washer is set for a soaking time of 1 minute.

Note: *Set the height of the needles; be sure the fluid can be taken up completely.*

Sample Dilution Guidelines

Determine the concentration of the target protein in the test sample and then select the optimal dilution factor to ensure the target protein concentration falls within the optimal detection range of the kit. Dilute the samples with the dilution buffer provided with the kit. Several dilution tests may be required to achieve the optimal results. The test samples must be well mixed with the dilution buffer. Standard and sample dilution should be performed before starting the experiment.

Note: *Dilution may be necessary to minimize matrix effects. However, if the target concentration in the sample is very low, the pre-treated sample can be added directly to the assay without dilution.*

Reagent Preparation

Bring all reagents and samples to room temperature 20 minutes before use (18 - 25°C). For repeated assays, please use only strips and standards required and store remaining reagents at the appropriate temperatures.

8. Assay Procedure

1. **Prepare the Standard Liquid**. Standard Liquid of low concentration is unstable, prepare fresh solution before use. Take 3 mL of Reconstitution Buffer (Solution 2) into 0 ppb bottle. Take 2 mL of Reconstitution Buffer (Solution 2) into 0.05 ppb bottle, 0.15 ppb bottle, 0.45 ppb bottle, 1.35 ppb bottle respectively. Take 3 mL of Reconstitution Buffer (Solution 2) into 4.05 ppb bottle.

   (1) Standard Liquid 6: Take 12 µL of High Concentrated Standard (1.0 ppm) into 4.05 ppb bottle, then mix fully. The concentration of Standard Liquid 6 is 4.05 ppb.

   (2) Standard Liquid 5: Take 1 mL of Standard Liquid 6 into 1.35 ppb bottle, then mix fully. The concentration of Standard Liquid 5 is 1.35 ppb.

   (3) Standard Liquid 4: Take 1 mL of Standard Liquid 5 into 0.45 ppb bottle, then mix fully. The concentration of Standard Liquid 4 is 0.45 ppb.

   (4) Standard Liquid 3: Take 1 mL of Standard Liquid 4 into 0.15 ppb bottle, then mix fully. The concentration of Standard Liquid 3 is 0.15 ppb.

   (5) Standard Liquid 2: Take 1 mL of Standard Liquid 3 into 0.05 ppb bottle, then mix fully. The concentration of Standard Liquid 2 is 0.05 ppb.

   (**6) Standard Liquid 1**: Use the Reconstitution Buffer directly. The concentration of Standard Liquid 1 is 0 ppb.
2. **Number:** Number the sample and standard in order (multiple well), and keep a record of standard wells and sample wells. Standard and Samples need test in duplicate.
3. **Add Sample:** add 50 µL of Standard or Sample per well, then add 50 µL Antibody Working Solution to each well, cover the plate with plate sealer, oscillate for 5 seconds gently to mix thoroughly, incubate at 37℃ for 30 minutes in shading light.

4. **Wash:** uncover the sealer carefully, remove the liquid of each well. Immediately add 350 µL of Wash Buffer (Solution 3) to each well and wash. Repeat wash procedure for 5 times, 30 seconds intervals/time. Invert the plate and pat it against thick clean with absorbent paper (If bubbles exist in the wells, clean tips can be used to prick them).
5. **HRP Conjugate:** add 100 µL of HRP conjugate to each well, incubate at 37℃ for 30 minutes in shading light.
6. **Wash:** repeat step 3.
7. **Color Development:** add 50 µL of Substrate Reagent A to each well, and then add 50 µL of Substrate Reagent B. Gently oscillate for 5 seconds to mix thoroughly. Incubate at 37℃ for 15 minutes with shading light (The reaction time can be extended according to the actual color change).
8. **Stop Reaction:** Add 50 µL of Stop Solution to each well, oscillate gently to mix thoroughly.
9. **OD Measurement**: determine the optical density (OD value) of each well at 450 nm (reference wavelength 630 nm) with a microplate reader. This step should be finished in 10 minutes after stop

9. Data Analysis

Calculation of Absorbance (%)

Absorbance (%) = A/A_0 x 100%

Where:

- **A** = Average absorbance of the standard or sample
- **A_0** = Average absorbance of the 0 ppb standard

Standard Curve Preparation and Calculation

To determine the concentration of Tetracyclines in samples:

- Plot the absorbance percentage (%) of each standard on the *y-axis* against the logarithm of concentration on the *x-axis* to obtain a semi-logarithmic standard curve.
- Locate the mean absorbance value of each sample on the curve to determine its corresponding concentration.

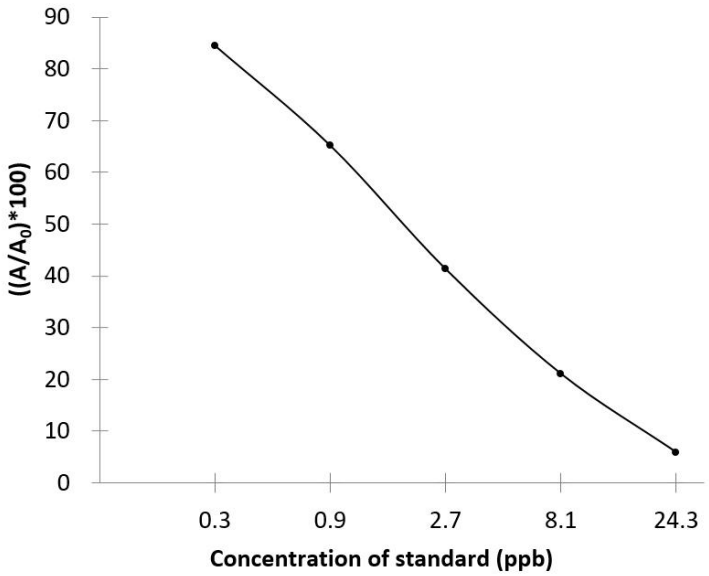
Note: *If the samples were diluted during pre-treatment, multiply the calculated concentration obtained from the standard curve by the dilution factor to determine the actual sample concentration*.

For improved efficiency and precision, it is recommended to use a professional analysis software or template for accurate and rapid evaluation of large sample batches.

10. Typical Data

Standard Curve

Results of a typical standard run of an ELISA kit are shown below. This standard curve was generated at our lab for demonstration purpose only. Each user should obtain their own standard curve as per experiment.



11. ELISA Troubleshooting

Problem	Possible Causes	Solutions
Standard curve without signal	Incorrect reagent order; Mixed components from different kits; Missing reagents.	Ensure correct reagent order and use components from the same kit. Verify all reagents are added.
Overflow OD	Mixed components from different kits; Over-concentrated working solution	Use correct components and prepare solutions at recommended concentrations.
Poor standard curve	Incorrect curve fitting model.	Try alternative curve fitting models.
Samples without signal	Sample concentration too low; Incompatible buffer; Incorrect preparation; Sample degradation or excessive freeze-thaw.	Reduce dilution or concentrate sample. Check buffer compatibility and follow proper preparation and storage.
High CV%	Precipitate formation; Unclean plate; Foaming; Uneven washing; Incomplete reagent mixing; Pipetting inconsistency.	Dilute samples if needed, avoid foaming, ensure uniform washing, mix reagents thoroughly, and use calibrated pipettes.
Low standard signal	Improperly reconstituted standards; Degraded standards; Incorrect pipetting; Expired kit; Improper storage; Over-dried wells.	Reconstitute standards properly, use fresh kits, follow storage recommendations, and prevent wells from drying.
Slow colour development	TMB not equilibrated; Incorrect microplate reader wavelength; Over-washing.	Pre-warm TMB (30 min at 37°C), confirm correct wavelength (450 nm), and follow recommended washing times.
High background	Insufficient washing; Contaminated wash buffer; Excess detection reagents; Delayed reading; TMB exposed to light.	Wash adequately, prepare fresh wash buffer, use correct reagent amounts, read results promptly, and incubate TMB in the dark.

Notes:

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.