Tetracyclines (TCs) ELISA Kit

Technical Manual

(ELISA)

Scan for more info.

Shenzhen Finder Biotech Co.,Ltd.
Web: www.szfinder.com
Tel: +86 0755 23499025 Email: techsupport@szfinder.com
Add: Building B12,Life Science Industrial Park, KuiyongSubdistrict, Dapeng New Area, Shenzhen,China

1 Principle and Application

This kit adopts the method of indirect competitive enzyme-linked immunoassay (ELISA) to detect Tetracyclines (TCs) in the sample such as tissue, honey and eggs. The kit is composed of Microtiter Plate coated with coupled antigens, HRP conjugate, antibodies, standards and other supporting reagents. During the detection, with adding standards or samples, the TCs in the samples will compete with the coupled antigens to combine with anti-TCs antibodies. After adding HRP conjugates, take coloration with TMB substrates. Absorbance value of the samples is a negative correlation with TCs content. Lastly, by comparing the obtained absorbance values with the standard curve, we can calculate the TCs content in the sample.

2 Technique Data

2.1 Kit Sensitivity: 0.05ppb (ng/mL)

2.2 Reactive Mode: 37°C, 30min ~ 30min ~ 15min

2.3 Detection Limits:

Sample	Detection Limits
Tissue, liver, eggs	2ppb
Honey	2ppb
Urine	0.5ppb
Milk	2ppb
Milk powder	4ppb

2.4 Cross-reaction Rate:

Tetracyclines 100%
Chlorotetracycline 16.7%
Oxytetracycline 107%
Doxycycline 4.2%

2.5 Sample Recovery Rate:

Sample	Recovery rate
Tissue, liver, egg	75±20%
Honey	80±20%
Urine	80±20%
Milk, Milk powder	75±20%

3 Composition of the Kit

Reagent	Specification
Microtiter Plate	8wells× 12strips
Antibody solution (blue cap)	1×5.5mL
High Standard: 1.0ppm (The Solution is volatile and needs to be sealed)	1×1.0mL
Standard: 0ppb, 0.05ppb, 0.15ppb, 0.45ppb, 1.35ppb, 4.05ppb (black cap; All are empty bottles, use it right after it was ready)	
HRP conjugate (red cap)	1×11mL
Substrate Reagent A (white cap)	1×6mL
Substrate Reagent B (black cap)	1×6mL
Stop Solution (yellow cap)	1×6mL
Concentrated Wash Buffer (20×) (white cap)	1×40mL
Concentrated Reconstitution Buffer (5×) (yellow cap)	1×50mL
Instruction	1
Adhesive Membrane	1
Sealed Bag	1

4 Materials Required but Not Supplied

4.1 Equipment: microplate reader, printer, grinder (for homogenizing solid samples), vortex mixer (for shake and mix), centrifuge, graduated transfer pipette, and balance with a division value of 0.01 g, constant temperature device;

4.2 Micropipette: single-channel (20-200μL and 100-1000μL), and multi-channel 300μL;

4.3 Reagents: Concentrated Hydrochloric Acid, Anhydrous methanol.

5 Experimental preparation

Restore all reagents and samples to room temperature (adjust to around 25°C) for more than 30 min before use. This is a crucial step to ensure there is no precipitation in the reagents.

5.1 Notice Before Sample Processing:

Please note that the labware must be clean. Use disposable pipette tips to avoid contamination of interference results.

5.2 Solution preparation:

Solution 1: Reconstitution Buffer

Dilute the Concentrated Reconstitution Buffer (5×) 5 times with deionized water (Concentrated Reconstitution Buffer (5×): deionized water=1:4). It can be stored at 4 °C for one month.

Solution 2: Working Wash Buffer

Dilute the concentrated wash buffer (20×) by a factor of 20 (Concentrated wash buffer/Deionized water= 1: 19).

Solution 3: Sample Extraction Solution

Take 4.3mL of concentrated hydrochloric acid, add deionized water, mix them well, bring to a constant volume of 50mL, and then add 450mL of anhydrous methanol.

5.3 Sample pretreatment steps:

5.3.1 Tissue, liver, egg treatment.

1) Accurately weigh 1g±0.05g of homogenized sample into a centrifuge tube, pipette 2mL of Sample Extraction Solution (Solution 3) to the tube, shake them for 2min to ensure complete dispersion and contact with the organic phase. Centrifuge the tube at 4000 rpm or higher at room temperature for 5min.

2) Retrieve 50μL of the upper layer solution (be cautious not to collect the white floating substance, as it contains more fat), and add it to 950μL of Reconstitution Buffer (Solution 1). Mix the solution thoroughly.

3) Take out 50μL for test.

Dilution times of the sample:40 Detection limits: 2ppb

5.3.2 Honey treatment.

1) Accurately weigh 1g±0.05g of honey sample into a centrifuge tube. Add 1mL of Sample Extraction Solution (Solution 3) to the tube. Shake them for 2min to ensure thorough dissolution of the honey.

2) Take out 50μL of the mixed solution obtained from the previous step, then add 950μL of Reconstitution Buffer (Solution 1) and mix well.

3) Take out 50μL for test.

Dilution times of the sample:40 Detection limits: 2ppb

5.3.3 Urine treatment.

1) Dilute the urine sample 10 times with the Reconstitution Buffer (Solution 1). If the urine sample is turbid, be sure to filter or centrifuge it for 10 minutes at 4000 rpm until a clear urine sample is obtained. Store any unused samples by freezing them for later use.

2) Take out 50μL for test.

Dilution times of the sample:10 Detection limits: 0.5ppb

5.3.4 Milk treatment.

1) Accurately aspirating 1mL of milk into a centrifuge tube. Add 2mL of Sample Extraction Solution (Solution 3) to the tube. Shake them for 2min and centrifuge it at room temperature for 5 minutes at 4000 rpm or higher.

2) Take out 50μL of the upper layer solution, then add 950 μL of Reconstitution Buffer (Solution 1) and mix well.

3) Take out 50μL for test.

Dilution times of the sample:40 Detection limits: 2ppb

5.3.5 Milk powder treatment.

1) Accurately weigh 1g±0.05g of milk powder into a centrifuge tube. Add 4mL of Sample Extraction Solution (Solution 3) to the tube. Shake them for 2min and centrifuge it at room temperature for 5 minutes at 4000 rpm or higher.

2) Take out 50μL of the upper layer solution, then add 950 μL of Reconstitution Buffer (Solution 1) and mix well.

3) Take out 50μL for test.

Dilution times of the sample:80 Detection limits: 4ppb

6 ELISA procedure

Place all reagents and samples to room temperature (adjust to around 25°C) for 30min. Gently shake the reagent bottles before use.

Take out the frame of the microplate along with the required number of wells. Then place the unused microplate wells into the sealed bag with the desiccant provided. Store the remaining kit in the refrigerator at 2-8°C.

Before starting the experiment, it is necessary to prepare the standards (Prepare the low-concentration standard when it is to be used as it is unstable).

Add 3mL of Reconstitution Buffer to the vial labeled as 0ppb. Add 2mL of Reconstitution Buffer to the vials labeled as 0.05ppb, 0.15ppb, 0.45ppb, and 1.35ppb, respectively. Add 3mL of Reconstitution Buffer to the vial labeled as 4.05ppb.

Standard 6: Pipette 12μL of the 1.0ppm **high standard** to the vial labeled as 4.05ppb (containing 3mL of Reconstitution Buffer). Close the vial tightly, mix thoroughly, and the concentration will be **4.05ppb.**

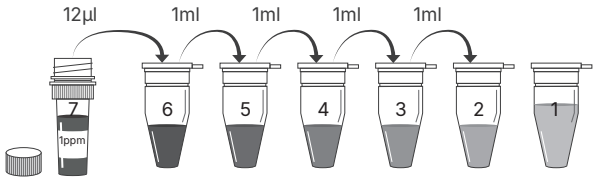
Standard 5: Pipette 1mL of **standard 6** to the vial labeled as 1.35ppb (containing 2mL of Reconstitution Buffer). Close the vial tightly, mix thoroughly, and the concentration will be **1.35ppb.**

Standard 4: Pipette 1mL of **standard 5** to the vial labeled as 0.45ppb (containing 2mL of Reconstitution Buffer). Close the vial tightly, mix thoroughly, and the concentration will be **0.45ppb.**

Standard 3: Pipette 1mL of **standard 4** to the vial labeled as 0.15ppb (containing 2mL of Reconstitution Buffer). Close the vial tightly, mix thoroughly, and the concentration will be **0.15ppb.**

Standard 2: Pipette 1mL of **standard 3** to the vial labeled as 0.05ppb (containing 2mL of Reconstitution Buffer). Close the vial tightly, mix thoroughly, and the concentration will be **0.05ppb.**

Standard 1: The Reconstitution Buffer can be directly used, and the concentration is **0 ppb.**



tube	7	6	5	4	3	2	1
	1.0ppm	4.05ppb	1.35ppb	0.45ppb	0.15ppb	0.05ppb	0ppb

Step 1: Number: Number the samples and standard corresponding microwells in order, make 2-well parallel trials for each sample and standard, and record the locations of sample wells and standard wells.

Step 2: Sample Incubation: Add 50μL of standard or sample into each numbered well, then add 50μL of antibody solution into each well. Finally, cover the Microtiter Plate with the adhesive membrane, shake gently by hand (or use a microplate shaker) for 5s and incubate for 30 min at 37°C.

Step 3: Washing: Uncover the adhesive membrane carefully, discard liquid in the wells, pipette 350μL of Working Wash Buffer (Solution 2) to every well, let stand for 30 seconds then drain, repeat 5 times. Invert the plate and tap it against a thick absorbent paper (or lint-free cloth), with a soft towel placed underneath. (Bubbles that are not removed after tapping dry can be punctured with a clean pipette tip).

Step 4: Enzyme incubation: Add 100μL of HRP conjugate per well, and incubate at 37°C in the dark for 30 minutes.

Step 5: Washing: Same as Step 3

Step 6: Color: Add 50μL of Substrate Reagent A to each well, then add 50μL of Substrate Reagent B. Shake gently by hand (or use a microplate shaker) for 5s, and allow to react for 15min at 37°C in the dark. (The reaction can be extended appropriately if the blue color is too pale.)

Step 7: Stop the reaction: Pipette 50μL of Stop Solution to each well, and shake gently by hand (or use a microplate shaker). The reaction would be stopped.

Step 8: Calculate: Determine the Optical Density (OD value; absorbance value) at 450nm (Reference wavelength 630nm) with a microplate reader. Finish this step within 10min after stop the reaction.

7 Interpretation of result

7.1 Calculate the percentage of absorbance value

$$\text{Percentage of absorbance value}(\%) = \frac{A}{A0} \times 100\%$$

A—the average OD value of the sample or standard;

A0—the average OD value of the 0ppb standard.

It is used to calculate the percentage absorbance of a standard or sample.

7.2 Draw the standard curve and calculate

Take absorbance percentage(A/A0) of standards as Y-axis and the corresponding log of standards concentration (ppb) as X-axis.

Draw the standard semi-log curves with X-axis and Y-axis.

Take absorbance percentage of samples substitute into standard curve, then can get the corresponding concentration from standard curve. Last, the resulting concentration values multiplied by the corresponding dilution times is the actual concentration of TCs of samples.

If professional analysis software of the kit is used for calculation, it is more convenient for accurate and rapid analysis of a large number of samples.

8 Attention

8.1 Before test, the reagents and samples should be balanced to room temperature (25°C). If below 25°C, it will lead to all the standard OD value on the low side.

8.2 In the washing process, dry wells may result in non-linear standard curves and undesirable reproducibility. Therefore, proceed to the next step immediately after washing.

8.3 Please mix the contents within the wells uniformly and wash the plate thoroughly. The reproducibility is largely determined by consistency of washing step.

8.4 During the incubation, cover microplates with adhesive membrane to avoid light.

8.5 Do not use kits that are overdue. Do not mix reagents with those from other lots.

8.6 Substrate Reagent A/B is colorless. If not, please discard.

8.7 If absorbance value of 0ppb is below 0.5（A450nm< 0.5), it means that the reagent may be metamorphic.

8.8 Stop solution is corrosives, please avoid contact with skin.

8.9 As the OD values of the standard curve may vary according to the conditions of actual assay performance (e.g. operator, pipetting technique, washing technique or temperature effects), the operator should establish a standard curve for each test.

8.10 For the mentioned sample, fast and efficient extraction methods are included in the kit description. Please consult technical support for the applicability if other sample need to be tested.

8.11 The kit is used for rapid screening of actual samples. If the test result is positive, the instrument method such as HPLC, LC/MS can be used for quantitative confirmation.

9 Storage conditions

The kit shall be stored at 2-8 °C. Avoid freezing.

Shelf Life: 12 months. The date of manufacture is presented in the label of the box.