Version 20-04/24

User's Manual

Chlortetracycline ELISA Kit

REF **DEIA-XYZ27**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

Chlortetracycline ELISA Test Kit is a competitive enzyme immunoassay for the quantitative analysis of Chlortetracycline in in honey, butter, whey, egg, cheese, milk, meat, meat products such as sausage, fish and shrimp.

General Description

1. High recovery (75-120%), rapid (less than 30 minutes), and cost-effective extraction methods.
2. High reproducibility.
3. A quick ELISA assay (less than 2 hours regardless of number of samples).

Principles of Testing

Chlortetracycline ELISA Test Kit is based on a competitive colorimetric ELISA assay. The drug of interest has been coated in the plate wells. During the analysis, sample is added along with the primary antibody specific for the target drug. If the target is present in the sample, it will compete for the antibody, thereby preventing the antibody from binding to the drug attached to the well. The secondary antibody, tagged with a peroxidase enzyme, targets the primary antibody that is complexed to the drug coated on the plate wells. The resulting color intensity, after addition of substrate, has an inverse relationship with the target concentration in the sample.

Reagents And Materials Provided

Chlortetracycline ELISA Test Kit has the capacity for 96 determinations or testing of 42 samples in duplicate (assuming 12 wells for standards). Return any unused microwells to the foil bag and reseal them with the desiccant provided in the original package.

Kit Contents	Amount	Storage
Chlortetracycline-Coated Plate	1 x 96-well plate (8 wells x 12 strips)	2-8°C
Chlortetracycline Standards: Negative control (white Cap tube) 0.5 ng/mL (yellow Cap tube) 1 ng/mL (orange Cap tube) 2 ng/mL (pink Cap tube) 4 ng/mL (purple Cap tube) 16 ng/mL (blue Cap tube) 100ng/mL (Spiking red Cap tube)	 0.2 mL 0.2 mL 0.2 mL 0.2 mL 0.2 mL 0.2 mL 1 mL	2-8°C
Chlortetracycline Antibody #1	6mL	2-8°C
HRP-Conjugated Antibody #2	12mL	
20X Wash Solution	30mL	
Stop Buffer	12mL	
30X Sample Dilution	30mL	
TMB Substrate	12mL	

Materials Required But Not Supplied

1. Microtiter plate reader (450 nm)
2. Vortex mixer, (e.g. Gneie Vortex mixer from VWR)
3. 10, 20, 100 and 1000 µL pipettes
4. Multi-channel pipette: 50-300 µL (Optional)

Storage

Store the kit at 2-8°C. The shelf life is 12 months when the kit is properly stored.* If you are not planning to use the kit for over 1 month, storing Chlortetracycline Standard Stock, Chlortetracycline Antibody #1 and HRP-Conjugated Antibody #2 at -20°C or in a freezer is recommended.

Specimen Collection And Preparation

Feed

1. Take 1 g of feed sample, add 5 mL of 1X Sample Dilution.
2. Vortex samples for 5 minutes using a multi-vortexer.
3. Centrifuge for 10 minutes at 4,000 x g.
4. Transfer 500µL of clear supernatant to a new tube containing 500µL of 1X Sample Dilution.
5. Vortex samples for 1 minute.
6. Use 100µL of the sample for the assay.

Note: Dilution factor: 10

Cheese/Egg/Sour Cream

1. Add 3mL of 2X Sample Dilution to 1 g of sample, vortex for 5 minutes in a multi-tube vortexer or shake 30 minutes on a shaker.
2. Centrifuge for 5 min at 4000 rpm.
3. Use 100µL per well in the assay.

Note: Dilution factor: 4

Honey

1. Weigh out 1 g of honey in a centrifuge vial.
2. Add 3mL 2X Sample Dilution.
3. Vortex for 5 minutes in a multi-tube vortexer or shake 15 minutes on a shaker.
4. Centrifuge for 5 min at 4000 rpm
5. Use 100 µL of the sample for the assay.

Note: Dilution factor: 4.

Meat/Meat Products Fish/Shrimp/Butter

1. To 1 g of homogenized sample, add 3 mL of 1X Sample Dilution.
2. Vortex for 5 minutes in a multi-tube vortexer or shake 15 minutes on a shaker.
3. Centrifuge for 5 minute at 4,000 x g.
4. Transfer 500µL of the supernatant to a new tube containing 500µL of distilled water.
5. Vortex for 1 minute.
6. Use 100µL per well in the assay.

Note: Dilution factor: 8

Milk/Yogurt/Curd

1. To 1 g of sample (or 1mL of liquid sample), Vortex for 5 minutes in a multi-tube vortexer or shake 15 minutes on a shaker.To 0.1 g of sample (or 0.1mL of liquid sample)add 0.9mL of distilled water.
2. Vortex for 5 minutes in a multi-tube vortexer or shake 15 minutes on a shaker.
3. Use 100µL per well in the assay.

Note: Dilution factor:10

Milk Powder

1. Reconstitute 1 g of dry milk powder with 10mL of deionized or distilled water. Mix well.
2. Use this solution as the starting sample.
3. To 1mL of sample, add 3 mL of 2XSample Dilution.
4. Vortex for 5 minutes in a multi-tube vortexer or shake 15 minutes on a shaker.
5. Use 100µL per well in the assay.

Note: Dilution factor: 4

Reagent Preparation

IMPORTANT: All reagents should be brought up to room temperature before use (1 – 2 hours at 20 – 25°C / 68 – 77°F); Make sure you read "Warnings and Precautions" section. Solutions should be prepared just prior

to ELISA test. All reagents should be mixed by gently inverting or swirling prior to use. Prepare volumes that are needed for the number of wells being run. Do not return the reagents to the original stock tubes/bottles. Using disposable reservoirs when handling reagents can minimize the risk of contamination and is recommended.

Preparation of 1X Sample Dilution

Mix 1 volume of 30X Sample Dilution with 29 volumes of distilled water.

Preparation of 2X Sample Dilution

Mix 1 volume of 30X Sample Dilution with 14volumes of distilled water

Preparation of 1X Wash Solution

Mix 1 volume of the 20X Wash Solution with 19 volumes of distilled water.

Preparation of 1X Standards

Mix 50 µL of the Standards with 450 µL of 1X Sample Dilution.

Assay Procedure

Special Notes for Optimal ELISA Performance

1) Allow the entire kit to equilibrate at room temperature for at least two hours before starting any ELISA assay.
2) Avoid light as much as possible during sample preparation and ELISA assay.
3) For plate washing steps: after addition of 250µL wash buffer to the wells, incubate the plate for 20 – 30 seconds; shake the plate gently before pouring out the wash buffer. Repeat this procedure for each of the 4 washes.
4) Pipette all reagents and samples very accurately, especially the samples, even if you must slow down while pipetting.

ELISA Testing Protocol

Label the individual strips that will be used and aliquot reagents as the following example:

Component	Volume per Reaction	24 Reactions
Chlortetracycline Antibody #1	50µL	1.2 mL
HRP-Conjugated Antibody #2	100µL	2.4 mL
1X Wash Solution	2.5 mL	60 mL
Stop Buffer	100 µL	2.4 mL
TMB Substrate	100 µL	2.4 mL

1. Add 100 µL of each Chlortetracycline Standards (Negative control, 0.05, 0.1, 0.2. 0.4, 1.6ppb) in duplicate into different wells (Add standards to plate only in the order from low concentration to high concentration).
2. Add 100µL of each sample in duplicate into different sample wells.
3. Add 50 µL of Antibody #1 and mix well by gently rocking the plate manually for 1 minute.
4. Incubate the plate for 30minutes at room temperature (20 – 25°C / 68 – 77℉).
5. Wash the plate 4 times with 250 µL of 1X Wash Solution. After the last wash, invert the plate and gently tap the plate dry on paper towels (Perform the next step immediately after plate washings. Do not allow the plate to air dry between working steps).

6. Add 100µL of 1X Antibody #2 solution. Incubate the plate for 30 minutes at room temperature (20 – 25°C / 68 – 77°F) (Avoid direct sunlight and cold bench tops during the incubation. Covering the microtiter plate while incubating is recommended).
7. Wash the plate 4 times with 250 of µL 1X Wash Solution. After the last wash, invert the plate and gently tap the plate dry on paper towels (Perform the next step immediately after plate washings. Do not allow the plate to air dry between working steps).
8. Add 100 µL of TMB Substrate to each well. Time the reaction immediately after adding the substrate. Mix the solution by gently rocking the plate manually for 1 minute while incubating , Incubate the plate for 15 minutes at room temperature (20 – 25°C / 68 – 77℉). (Do not put any substrate back to the original container to avoid any potential contamination. Covering the microtiter plate while incubating is recommended).
9. After incubating,add 100 µL of Stop Buffer to stop the enzyme reaction.
10. Read the plate as soon as possible following the addition of Stop Buffer on a plate reader with 450 nm wavelength (Before reading, use a lint-free wipe on the bottom of the plate to ensure no moisture or fingerprints interfere with the readings).

Calculation

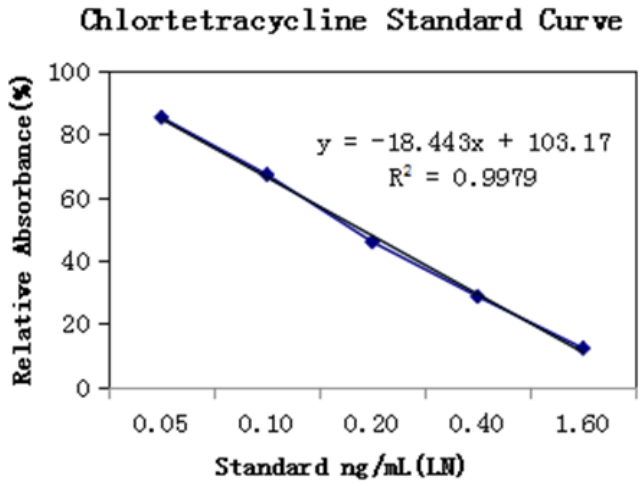
A standard curve can be constructed by plotting the mean relative absorbance (%) obtained from each reference standard against its concentration in ng/mL on a Logarithm curve.

Relative absorbance (%) = absorbance standard (or sample) x 100/absorbance zero standard

When use computing software, recommends Log/Log standard curves.

Typical Standard Curve

The following figure is a typical chlortetracycline standard curve.



Specificity

Chlortetracycline

Precautions

1. The standards contain chlortetracycline. Handle with particular care.
2. Do not use the kit past the expiration date.
3. Do not intermix reagents from different kits or lots except for components with the same part No's within their expiration dates. ANTIBODIES AND PLATES ARE KIT-AND LOT-SPECIFIC.
4. Try to maintain a laboratory temperature of 20–25°C (68°–77°F). Avoid running assays under or near air vents, as this may cause excessive cooling, heating and/or evaporation. Also, do not run assays in direct sunlight, as this may cause excessive heat and evaporation. Cold bench tops should be avoided by placing several layers of paper towel or some other insulation material under the assay plates during incubation.
5. Make sure you are using only distilled or deionized water since water quality is very important.
6. When pipetting samples or reagents into an empty microtiter plate, place the pipette tips in the lower corner of the well, making contact with the plastic.
7. Incubations of assay plates should be timed as precisely as possible. Be consistent when adding standards to the assay plate. Add your standards first and then your samples.
8. Add standards to plate only in the order from low concentration to high concentration as this will minimize the risk of compromising the standard curve.
9. Always refrigerate plates in sealed bags with a desiccant to maintain stability. Prevent condensation from forming on plates by allowing them equilibrate to room temperature (20 – 25°C / 68 – 77℉) while in the packaging.