REAGEN™

Chlortetracycline ELISA Test Kit

RND99050

Product Description

REAGEN™ Chlortetracycline ELISA Test Kit provides a competitive enzyme immunoassay for the quantitative analysis of Chlortetracycline in honey, milk, meat and urine.

The unique features of the kit are:

- Rapid , high recovery (75-105%), and cost-effective extraction methods.
- High sensitivity (0.05 ng/g or ppb) and low detection limit.
- High reproducibility.
- A quick ELISA assay (less than 2 hours regardless of number of samples).

Procedure Overview

REAGEN™ 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.

Kit Contents, Storage and Shelf Life

REAGEN™ 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. Store the kit at 2-8°C. The shelf life is 12 months when the kit is properly stored.

Kit Contents	Amount	Storage
Chlortetracycline-coated Microtiter Plate	1x 96-well plate(8wells x12 strips)	2-8°C
Chlortetracycline Standards stock (450ng powder)	3	-20°C
Empty Vials for standards:		
Negative control (white cap tube)	1	
0.05 ng/mL (yellow cap tube)	1	
0.1ng/mL (orange cap tube)	1	2-8°C
0.2 ng/mL (pink cap tube)	1	
0.4 ng/mL (purple cap tube)	1	
0.8 ng/mL (blue cap tube)	1	
50 ng/mL(red cap tube)	1	2-8°C
Chlortetracycline Antibody #1	12ml	2-8°C
100X HRP-Conjugated Antibody #2	0.3mL	
Antibody #2 Diluent	20 mL	
20X Wash Solution	30 mL	
Stop Buffer	14 mL	
TMB Substrate	10mL	
5X OXYTET Extraction Buffer (optional, ask from local distributor)	2 X 25 mL	
Standard Diluent	28 mL	

If you are not planning to use the kit for over 1 months, store Chlortetracycline Antibody #1 and 100X HRP-Conjugated Antibody #2 at -20°C or in a freezer.

Sensitivity

Sample Type	**Detection Limit** (ng/g or ppb)
Honey	1.25
Meat/Fish/Shrimp	1
Milk	2.5
Milk powder	22.5
Serum	2
Urine	2

Specificity

Analytes	**Cross-Reactivity** (%)
Chlortetracycline	100
Oxytetracycline	91
Minocycline	73
Demeclocycine	39
Tetracycline	119
Doxycycline	60

Required Materials Not Provided With the Kit

- Microtiter plate reader (450 nm)
- Vortex mixer (e.g. Gneie Vortex mixer from VWR)
- 10, 20, 100 and 1000 µL pipettes

- Multi-channel pipette: 50-300 µL (Optional)
- 10Mmpbs buffer: 0.24g KH_2PO_4 +1.44 g Na_2HPO_4+ 8gNaCl, + 0.2gKCl, adjust pH to 7.4 with NaOH, fill up to 1000 mL with distilled water

Warnings and Precautions

- The standards contain Chlortetracycline. Handle with particular care.
- Do not use the kit past the expiration date.
- 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. Make sure that the antibody #2 and diluent are mixed in correct volumes.
- 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.
- Make sure you are using only distilled or deionized water since water quality is very important.
- 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.
- 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.
- 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.
- 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°F) while in the packaging.

SAMPLE PREPARATION

Be sure samples are properly stored. In general, samples should be refrigerated at 2-4°C for no more than 1-2 days. Freeze samples to a minimum of -20°C if they need to be stored for a longer period. Frozen samples can be thawed at room temps (20 – 25°C / 68 – 77°F) or in a refrigerator before use.

Preparation of 1X OXYTET Extraction Buffer

Mix 1 volume of 5X OXYTET Extraction Buffer with 4 volumes of distilled water.

Honey

1. Weigh out 0.5g of honey in a screw-top glass vial(50ml).
2. Add 12 mL 10mM PBS buffer.
3. Put the solution in an ultrasonic bath for 5 min and vortex for 2 min.
4. Use 75 µL of the sample for the assay.

   Note: Dilution factor: 25.

Meat/Fish/Shrimp

1. Add 3 ml of 1X OXTET Extraction Buffer to 1g of homogenized meat, vortex for 10 min in a multi-tube vortexer.
2. Centrifuge at 4,000 x g for 10 minutes at room temperature (20 – 25°C / 68 – 77°F).
3. Transfer 200 µL of the supernatant into a new vial containing 800 µL of 10mm PBS buffer, PH 7.4,vortex for 30 seconds.
4. Use 75 µL per well for the assay.

   Note: Dilution factor: 20.

Milk

1. For regular milk with fat, centrifuge 1.5 mL of the cold milk sample at 10000 x g for 10 minutes. Discard the upper fat layer.(For fat-free milk, skip this step).
2. Take 0.1mL of the milk sample and add 4.9 mL of 10 mM PBS buffer, pH 7.4. Vortex the tube for 30 seconds.
3. Use 75 µL per well for the assay.

   Note: Dilution factor: 50

Milk powder

1. Weigh out 1 g of milk powder in a centrifugal glass vial, add 9 mL of distilled water and dissolve by shaking.
2. Centrifuge at 10000 rpm for 10 minutes, discard the upper lipid layer.
3. Take 200 µL of the milk sample and add 4.8 mL of 10 mM PBS buffer, pH 7.4. Vortex the tube for 30 seconds.
4. Use 75 µL of the sample for the assay.

   Note: Dilution factor: 225

Serum/Plasma

1. Take 0.2ml of the sample, centrifuge at 4000g for 5 min.
2. Take 0.1ml of the supernatant, add 3.9ml of 10mm PBS buffer ,PH7.4
3. Vortex for 1 min.
4. Use 75 µL per well for the assay.

   Note: Dilution factor:40

Urine

1. Centrifuge 1.5ml of the urine sample at 4,000 x g for 5 minutes.
2. Take 0.5ml of the supernatant,add 19.5ml of 10mm PBS buffer, PH 7.4
3. Vortex for 1 min.
4. Use 75 µL per well for the assay.

   Note: Dilution factor: 40

CHLORTETRACYCLINE ELISA TEST KIT PROTOCOL

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 on page 3. 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.

1. Preparation of Chlortetracycline Work Standards

   The Chlortetracycline standard is provided as 450 ng stock. Add 1.5 mL of standard diluent to the stock vial and mix by vortexing for 1 minute to obtain 300 ppb stock vial. To make work standards, serially dilute the 300 ppb standard stock vial with standard diluent, mix well by vortexing each work standard tube for 30 seconds.

Work standards	Chlortetracycline source	Volume of Source Chlortetracycline	Volume of standard diluent
50ppb	300ppb	250µL	1250µL
0.8ppb	50ppb	20µL	1230µL
0.4ppb	0.8ppb	500µL	500µL
0.2ppb	0.4ppb	500µL	500µL
0.1ppb	0.2ppb	500µL	500µL
0.05ppb	0.1ppb	500µL	500µL
Negative control	N/A	0µL	500µL

   Note: The 300 ppb stock vial and the work standards must be freshly prepared before the ELISA test and these standards can be used within the same day. After use the work standard, empty the standard vials and store them in 4°C. These empty vials can be re-used for next time standard preparation.

2. Preparation of 1X Wash Solution

   Mix 1 volume of 20X Wash Buffer concentrate with 19 volumes of distilled water.

3. Preparation of 1X HRP-Conjugated Antibody #2

   Mix 1 volume of 100X Antibody #2 with 99 volumes of Antibody #2 Diluent.

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	100 µL	2.4 mL
1X HRP-Conjugated Antibody #2	150 µL	3.6 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 75 µL of each Chlortetracycline Standards in duplicate into different wells (☞ Add standards to plate only in the order from low concentration to high concentration).
2. Add 75 µL of each sample in duplicate into different sample wells.
3. Add 100 µL of Chlortetracycline Antibody #1 and mix well by gently rocking the plate manually for 1 minute.
4. Incubate the plate for 30 minutes at room temperature (20 – 25°C / 68 – 77°F).
5. Wash the plate 3 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 150 µL 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 3 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).
8. Add 100 µL of TMB substrate. Time the reaction immediately after adding the substrate. Mix the solution by gently rocking the plate manually for 1 minute while incubating (Do not put any substrate back to the original container to avoid any potential contamination. Any substrate solution exhibiting coloration is indicative of deterioration and should be discarded. Covering the microtiter plate while incubating is recommended).
9. After incubating for 15 minutes at room temperature (20 – 25°C / 68 – 77°F), 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).

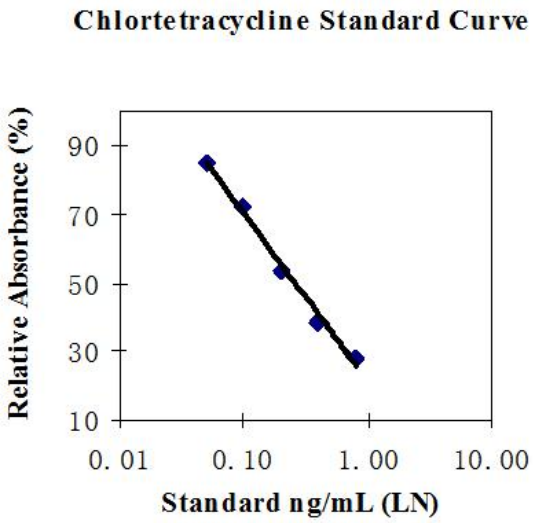
Chlortetracycline Concentration Calculations

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 logarithmic curve.

$$\text{Relative absorbance (\%)} = \frac{\text{absorbance standard (or sample) x 100}}{\text{absorbance zero standard}}$$

Use the mean relative absorbance values for each sample to determine the corresponding concentration of the tested drug in ng/mL from the standard curve.

The following figure is a typical Chlortetracycline standard curve.



REAGEN LLC

Add.: 102CommerceDr.Unit8,Moorestown,NJ08057

Tel.:856-727-0250 FAX.:856-727-0251

E-mail:**reagenkits@gmail.com** **reagenllc@gmail.com**

Web.:www.reagen.us