REAGEN™

Oxytetracycline ELISA Test Kit

RND99049

Product Description

REAGEN™ Oxytetracycline ELISA Test Kit provides a competitive enzyme immunoassay for the quantitative analysis of Oxytetracycline in hony, milk, meat fish, shrimp and feed.

The unique features of the kit are:

- Rapid , high recovery (80-115%), and cost-effective extraction methods.
- High sensitivity (0.15 ng/g or ppb) and low detection limit of sample).
- High reproducibility.
- A quick ELISA assay (less than 2 hours regardless of number of samples).

Procedure Overview

The method 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™ Oxytetracycline 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
Oxytetracycline-coated Microtiter Plate	1x 96-well plate (8wells x12 strips)	2-8°C
Oxytetracycline Standards stock:(450ng powder)	3	-20°C
Empty Vials for standards:		
Negative control (white cap tube)	1	
0.15 ng/mL (yellow cap tube)	1	
0.375ng/mL (orange cap tube)	1	
0.75 ng/mL (pink cap tube)	1	
1.5 ng/mL (purple cap tube)	1	
4.5 ng/mL (blue cap tube)	1	
Oxytetracycline Spiking Stock (2000 ng Powder)	1	
Standards diluent	28ml	2-8°C *
Oxytetracycline Antibody #1	18ml	
100X HRP-Conjugated Antibody #2	0.3mL	
Antibody #2 Diluent **	20 mL	
20X Wash Solution **	28 mL	
Stop Buffer **	14 mL	
TMB Substrate **	12 mL	
5x OXYTET Ext. buffer	2x 25mL	
2X OXYTET Balance Buffer Concentrate	4 mL	
10X TET Sample Diluent	28 mL	

* If you are not planning to use the kit for over 1 months, store Oxytetracycline Antibody #1 at 4℃ and 100X HRP-Conjugated Antibody #2 at -20°C or in a freezer.

Sensitivity

Sample Type	**Detection Limit** (ng/g or ppb)
Egg/Cheese/Sour Cream	1.5
Honey	3.0
Feed	9
Meat/Meat Produts/Fish/Shrimp/Butter	1.5
Milk/Sour Milk/Yogurt/Curd	1.5
Reconstituted Milk Powder	1.5
Reconstituted Whey Powder	1.5

Specificity

Analytes	**Cross-Reactivity** (%)
Oxytetracycline	100
Chlortetracycline	108
Minocycline	79
Demeclocycine	37
Tetracycline	124
Doxycycline	62

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)
- n-hexane
- 1M $NaHCO_3$ in deionized water
- 1×PBS: To make 1 L, dissolve 0.24 g KH_2PO_4 + 1.44 g Na_2HPO_4 + 8 g NaCl, + 0.2 g KCl in deionized water to a final volume of 1 L. *(Ask your local distributor about ordering a pre-made 10X PBS solution.)*

Warnings and Precautions

- The standards contain Oxytetracycline. 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.

1. **Preparation of 1X OXYTET Extraction Buffer：**
   Mix 1 volume of 5X OXYTET Extraction Buffer with 4 volumes of distilled water.
2. **Preparation of Oxytetracycline Spiking Standard**
   The oxytetracycline spiking is provided as 2000 ng powder stock. Take out the stock powder, add 1.0 mL of Standard Diluent. Vortex vigorously for 2 minutes, then leave this solution at room temperature at dark for 15 minutes. Then vortex vigorously again for 1 minute and leave it at room temperature at dark for another 15 minutes. Vortex the solution for about 30 seconds for a third time, and then transfer this 2000 ppb stock solution to a **dark plastical** tube and store it at -20℃ for any future use.
3. **Preparation of 1X TET Sample Diluent**
   Mix 1 volume of 10c TET Sample Diluent with 9 volumes of distilled water.
4. **Preparation of 1X OXYTET Balance Buffer**
   Combine 1.5 mL of TET Balance Buffer Concentrate and 1.5 mL of deionized water.
5. **Preparation of 1X OXYTET Feed Balance Buffer A**
   To make 10 mL: Combine 9 mL of 1X TET Sample Diluent, 0.8 mL deionized water, and 0.2 mL of 2X TET Balance Buffer Concentrate. Vortex well to dissolve.

Cheese / Egg / Cream

1. To 1 g of cheese or egg sample, add 3 mL of 1X OXYTET Extraction Buffer.
2. Vortex for 10 minutes in a multi-tube vortexer or shake 30 minutes on a shaker.
3. Centrifuge for 10 minutes at 4,000 x g.
4. Transfer 200 µL of the supernatant to a new tube containing 25 µL of 1X OXYTET Sample Balance Buffer. Swirl the tube. Then add 275 µL of 1X TET Sample Diluent.
5. Vortex for 1 minute.
6. Use 75 µL per well in the assay.
   Note: *Dilution factor: 10*

Honey

1. Weigh out 0.5 g of honey in a centrifuge vial.Add 3.5 mL of 1X OXYTET Extraction Buffer and 1 mL of n-hexane.Ensure the sample is fully dissolved in this buffer by heating for 5 – 10 minutes at 50 °C.
2. Vortex for 10 minutes in a multi-tube vortexer or shake 30 minutes on a shaker.
3. Centrifuge for 10 minutes at 4,000 x g.
4. Transfer 200 µL of the supernatant to a new tube containing 25 µL of 1X OXYTET Sample Balance Buffer. Swirl the tube. Then add 275 µL of 1X TET Sample Diluent.

5. Vortex for 1 minute.
6. Use 75 µL per well in the assay.
   Note: Dilution factor:20.

Meat/ Meat Products/Fish/Shrimp/Butter

1. To 1 g of homogenized sample, add 3 mL of 1X OXYTET Extraction Buffer and 1 mL n-hexane,For shrimp samples ONLY: DO NOT add n-hexane.
2. Vortex for 10 minutes in a multi-tube vortexer or shake 30 minutes on a shaker.
3. Centrifuge for 10 minute at 4,000 x g.
4. Transfer 200 µL of the supernatant to a new tube containing 25 µL of 1X OXYTET Sample Balance Buffer. Swirl the tube. Then add 275 µL of 1X TET Sample Diluent.
5. Vortex for 1 minute.
6. Use 75 µL per well in the assay.
   Note: Dilution factor: 10.To detect Oxytetracycline in the range of 15 – 450 ppb: After Step 5, transfer 100 µ L of this sample to 900 µ L of 1X TET Sample Diluent.Vortex for 1 minute. The dilution factor will be 100.

Feed

1. To 1 g of a fully ground sample add 4 mL of 1X OXYTET Extraction Buffer.
2. Vortex for 10 minutes in a multi-tube vortexer or shake 30 minutes on a shaker.
3. Centrifuge for 10 minute at 4,000 x g.
4. Transfer 50 µL of the supernatant to a new tube containing 700 µL of 1X OXYTET Feed Balance Buffer A.
5. Vortex for 2 minutes.
6. Use 75µL per well in the assay.
   Note: Dilution factor: 60

Milk/ Soured Milk / Yogurt / Curd / Soured Cream

1. To 1 g of sample *(or 1 mL of liquid sample)*, add 3 mL of 1X OXYTET Extraction Buffer.
2. Vortex for 10 minutes in a multi-tube vortexer or shake 30 minutes on a shaker.
3. Centrifuge for 10 minutes at 4,000 x g.
4. Transfer 200 µL of the supernatant to a new tube containing 25 µL of 1X OXYTET Sample Balance Buffer. Swirl the tube. Then add 275 µL of 1X TET Sample Diluent.
5. Vortex for 1 minute.
6. Use 75 µL per well in the assay.
   Note: Dilution factor: 10

Milk powder

1. Reconstitute 1 g of dry milk powder with 9 mL of deionized or distilled water. Mix well.
2. To 1 mL of sample, add 3 mL of 1X OXYTET Extraction Buffer.
3. Vortex for 10 minutes in a multi-tube vortexer or shake 30 minutes on a shaker.
4. Centrifuge for 10 minutes at 4,000 x g.
5. Transfer 200 µL of the supernatant to a new tube containing 25 µL of 1X OXYTET Sample Balance Buffer. Swirl the tube. Then add 275 µL of 1X TET Sample Diluent
6. Vortex for 1 minute.
7. Use 75 µL per well in the assay.
   Note: Dilution factor: 10

Whey powder

1. Reconstitute 1 g of dry whey powder with 12 mL of deionized or distilled water. Mix well.
2. To 1 mL of sample, add 75 µL of 1 M $NaHCO_3$ (aq). Swirl the tube, then add 3 mL of 1X OXYTET Extraction Buffer.
3. Vortex for 10 minutes in a multi-tube vortexer or shake 30 minutes on a shaker.
4. Centrifuge for 10 minutes at 4,000 x g.
5. Transfer 200 µL of the supernatant to a new tube containing 25 µL of 1X OXYTET Sample Balance Buffer. Swirl the tube. Then add 275 µL of 1X TET Sample Diluent.
6. Vortex for 1 minute.
7. Use 75 µL per well in the assay.
   Note: Dilution factor: 10

Oxytetracycline 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 Oxytetracycline Work Standards

The Oxytetracycline standard is provided as a 450 ng lyophilized powder. To this powder, add 1.5 mL of Standard Diluent to obtain a 300 ppb solution. Vortex the solution vigorously

for 2 minutes and leave it at room temperature for 10 minutes. Repeat this procedure three times to ensure that all of the powder has dissolved. Transfer the solution to a dark plastic vial and store it at -20 °C for any future use. To make the working standards, serially dilute the 300 ppb stock solution with Standard Diluent. Vortex each standard for at least 30 seconds before continuing to the next dilution.

Work standards	Oxytetracycline source	Volume of Oxytetracycline	Volume of Standards diluent
4.5ppb	300ppb	30µL	1970µL
1.5ppb	4.5ppb	500µL	1000µL
0.75ppb	1.5ppb	500µL	500µL
0.375ppb	0.75ppb	500µL	500µL
0.15ppb	0.375ppb	500µL	750µL
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. *When finished, empty the standard vials. These empty vials can be re-used for the next preparation of the working standards.*

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.

4. **Special Notes for Optimal ELISA Performance**

a) Allow the entire kit to equilibrate at room temperature for at least two hours before starting any ELISA assay.

b) Avoid light as much as possible during sample preparation and ELISA assay.

c) For plate washing steps: after addition of 250 µL 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 three washes.

d) 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
Oxytetracycline 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 Oxytetracycline 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 Oxytetracycline Antibody #1 and mix well by gently rocking the plate manually for 1 minute.
4. Incubate the plate for 50 minutes at room temperature (20 – 25°C / 68 – 77°F).
5. Wash 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 20 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 plate 3 times with 250µL 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).

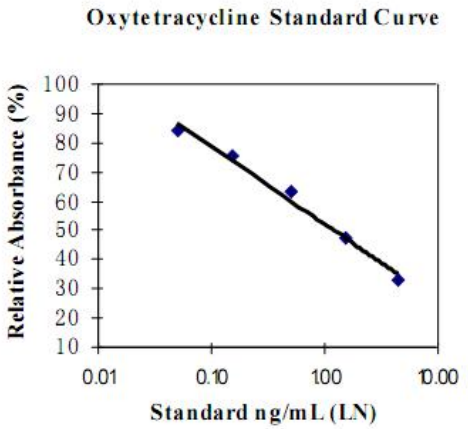
Oxytetracycline Concentration Calculations

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 Oxytetracycline standard curve.



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