Read instructions carefully before starting test

Veratox®

for Tetracycline

Refrigerate at 2–8°C (35–46°F) • Do not freeze

TETRACYCLINE

Tetracycline is a class of antibiotics, which has broad spectrum use. Tetracyclines are frequently used in animal production for their excellent antibacterial and pharmacokinetic properties, allowing for effective treatment of disease.

INTENDED USE

Veratox® for Tetracycline provides a rapid, high recovery, cost-effective and competitive enzyme immunoassay for the quantitative analysis of Tetracycline in muscle.

INTENDED USER

Veratox for Tetracycline enables regulatory agencies, food manufacturers and processors to detect Tetracycline in a variety of sample types in order to satisfy concerns about food safety or label claims.

ASSAY PRINCIPLES

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.

STORAGE REQUIREMENTS

Store kit at 2–8°C (35–46°F). If the kit is not used within a month, some components should be stored at frozen temperatures. The shelf life of the kit is 12 months when properly stored.

MATERIALS PROVIDED

Kit Contents	Amount	Storage
Tetracycline-coated Plate	1 x 96-well plate (8 wells x 12 strips)	2–8ºC
Tetracycline Standard: 0 ng/mL (white cap tube) 0.05 ng/mL (yellow cap tube) 0.15 ng/mL (orange cap tube) 0.4 ng/mL (pink cap tube) 0.8 ng/mL (purple cap tube) 1.6 ng/mL (blue cap tube) 100 ng/mL (spiking, red cap tube)	 1.2 mL 1.2 mL 1.2 mL 1.2 mL 1.2 mL 1.2 mL 1.2 mL	2–8ºC*
Tetracycline Antibody #1*	12mL	2–8ºC*
100X HRP-Conjugated Antibody #2*	300 µL	2–8ºC*
Antibody #2 Diluent**	20 mL	2–8ºC
20X Wash Solution**	28 mL	2–8ºC
Stop Buffer**	14 mL	2–8ºC
TMB Substrate**	12 mL	2–8ºC
5X OXYTET Extraction Buffer	2 x 25 mL	2–8ºC
10X TET Sample Diluent	28 mL	2–8ºC
TET Balance Buffer Concentrate	5 mL	2–8ºC

* If the kit will be unused for over 1 month, store Tetracycline standards, Tetracycline Antibody #1 and 100X HRP-Conjugated Antibody #2 at -20 ºC or in a freezer.

** These components (within their expiry) can be used interchangeably with other Veratox for Tetracycline kits.

MATERIALS RECOMMENDED BUT NOT PROVIDED

1. Microtiter plate reader with a 450 nm filter (Neogen item 9303)
2. 20–200 µL pipettes (Neogen item 9276)
3. Multichannel pipette: 50–300 µL (optional) (Neogen item 9385)
4. 1000 µL pipettor (Neogen 9337)
5. Vortex Mixer (Neogen 9494)
6. Centrifuge 4,000 x *g*
7. Timer (Neogen item 9426)
8. Wash bottle (Neogen item 9400)
9. Paper towels or equivalent absorbent material
10. Veratox software (Neogen item 9305)
11. Lab station (Neogen item 9481)
12. Distilled or deionized water
13. Blender or food processor to homogenize sample

PRECAUTIONS

Neogen strongly recommends that you read the following precautions to ensure your full awareness of ELISA techniques and other details you should pay close attention to when running the assays. More information can also be found in the Technical Solutions & Troubleshooting section. As the kit and manual are periodically revised, please follow the protocol included with the kit. If you need further assistance, you may contact Neogen at 800/234-5333 or 517/372-9200.

1. The standards contain Tetracycline. 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 numbers 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 under the assay plates during incubation.
5. Make sure you are using only distilled or deionized water since water quality is very important.
6. 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.
7. Follow proper pipetting techniques, including priming tips by filling and dispensing solution once before use.
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 to equilibrate to room temperature (20–25ºC / 68–77ºF) while in the sealed bag.

Tips 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 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.

SAMPLE PREPARATION

Be sure samples are properly stored. In general, samples should be refrigerated at 2–4°C for no more than 48 hours. 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 temperature (20–25ºC / 68–77ºF) or in a refrigerator before use.

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

B. Part 1: Preparation of 1X TET Balance Buffer
Mix 1 volume of 10X TET Sample Diluent with 9 volumes of distilled water.

Part 2: Preparation of 1X TET Sample Balance Buffer
Combine 750 µL of TET Balance Buffer Concentrate and 29.25 mL of 1X TET Sample Diluent. Mix very well.

SAMPLE EXTRACTION

Muscle

1. Homogenize tissue to the consistency of a purée. Weigh 1 g (± 0.05 g) of tissue into a 15 mL conical tube. Force all tissue near the bottom of the tube.
2. Incubate the sample for **30 minutes** at 45°C.
3. Add 3.0 mL of 1X OXYTET Extraction Buffer. Close the tube and shake vigorously until all tissue is immersed and broken up in the buffer. Vortex for 1 minute.
4. Add 1 mL (± 0.1 mL) of n-hexane. Vortex for 1 minute.
5. Incubate sample for **10 minutes** at 45°C.
6. Vortex sample manually or using a multi-tube vortexer for **5 minutes** ± 1 minute.
7. Centrifuge sample for **10 minutes** at maximum speed (~ 4,000 x *g*).
8. Using a pipette tip to carefully pierce through the hexane layer and interphase, transfer 1 mL of the lower aqueous layer into a new transfer tube. Alternatively, the upper hexane layer and interphase can be completely removed by vacuum aspiration or pipetting.
   NOTE: Ensure that a minimal amount of particles from the interphase are transferred to the new tube. Any particles may interfere with ELISA readings.
9. Transfer 200 µL of the lower aqueous layer to a new tube containing 300 µL of 1X TET Sample Balance Buffer.
10. Vortex for 2 minutes. Incubate for **15 minutes** at room temperature (20–25ºC / 68–77ºF) to allow pH equilibration. Vortex for an additional 1 minute.
11. Use 75 µL per well in the assay.

    NOTE: Dilution factor: 10.

REAGENT PREPARATION

IMPORTANT: All reagents should be brought up to room temperature before use (1–2 hours at 20–25ºC / 68–77ºF). Make sure you read the Precautions section on page 3. Solutions should be prepared prior to running the 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.

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

B. Preparation of 1X HRP-Conjugated Antibody #2
Mix 1 volume of the 100X HRP-Conjugated Antibody #2 with 99 volumes of Antibody #2 Diluent.

TEST PROCEDURE

Label the individual strips that will be used and prepare reagent aliquots based on the number of reactions:

Component	Volume Per Reaction	24 Reactions
Tetracycline 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 Tetracycline Standard in duplicate into different wells.
   NOTE: Add standards to plate in order from low concentration to high concentration (white, yellow, orange, pink, purple, blue).
2. Add 75 µL of each sample in duplicate into different sample wells.
   NOTE: Because of the nature of some samples, a precipitate may form after sample preparation. It is therefore required to vortex the sample thoroughly immediately before adding to each well.
3. Add 100 µL of Antibody #1 and mix well by sliding back and forth on a flat surface and tapping against a hard force point for 1 minute. Incubate for **30 minutes** at room temperature (20–25ºC / 68–77ºF).
   NOTE: Hard force point can be created by pressing fingers against the flat surface.
4. Manually mix the plate for **1 minute** by sliding back and forth on a flat surface against a hard force point. Incubate for **15 minutes** at room temperature (20–25ºC / 68–77ºF).
5. Following the 15 minute incubation, manually mix the plate again for an addtional **1 minute** by sliding back and forth on a flat surface and tapping against a hard force point. Incubate again for **10 minutes** at room temperature (20–25ºC / 68–77ºF).
   NOTE: Total incubation time for steps 3–5 is 55 minutes.
6. Shake out the contents of the wells into a waste container. Wash each well with 250 µL of 1X Wash Solution and dump out, repeat the wash step 3 times. After the last wash, invert the plate and gently tap the plate dry on paper towels.
   NOTE: Perform the next step immediately (within 1 minute) after drying the plate. Do not allow the plate to air dry between working steps.
7. Add 150 µL of the 1X Antibody #2 solution and mix well by sliding back and forth on a flat surface and tapping against a hard force point for 1 minute. Incubate the plate for **20 minutes** at room temperature (20–25ºC / 68–77ºF).
   NOTE: Avoid direct sunlight and cold bench tops during the incubation. Covering the microtiter plate while incubating is recommended.
8. Shake out the contents of the wells into a waste container. Wash each well with 250 µL of 1X Wash Solution and dump out, repeat the wash step 3 times. After the last wash, invert the plate and gently tap the plate dry on paper towels.
   NOTE: Perform the next step immediately (within 1 minute) after drying the plate. Do not allow the plate to air dry between working steps.
9. Add 100 µL of TMB Substrate to each well. Incubate the plate for **15 minutes** at room temperature (20–25ºC / 68–77ºF). Time the reaction immediately after adding the substrate. Mix the solution by sliding back and forth on a flat surface for **1 minute** while incubating.
   NOTE: Do not put any substrate back into the original container to avoid any potential contamination. Covering the microtiter plate while incubating is recommended.
10. After incubation, add 100 µL of Stop Buffer to stop the enzyme reaction.
11. Read the plate as soon as possible following the addition of Stop Buffer on a plate reader with a 450 nm primary filter.
    NOTE: Before reading, use a lint-free wipe on the bottom of the plate to ensure no moisture or fingerprints interfere with the readings.

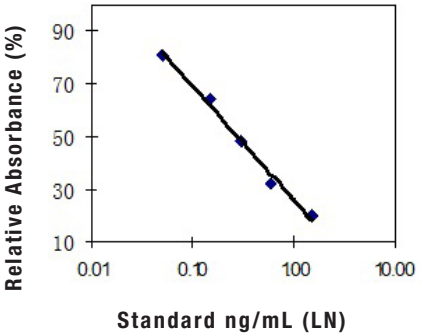
INTERPRETATION OF RESULTS

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.

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

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. Veratox software is available upon request to evaluate the results. Please contact Neogen for further information. The following figure is a typical Tetracycline standard curve:

Tetracycline Standard Curve



SENSITIVITY (Assay Range)

Sample Type	Assay Range (ng/g or ppb)
Muscle	0.5 – 16

SPECIFICITY (Cross-Reactivity)

Analytes	Cross-Reactivity (%)
Tetracycline (TET)	100
Chlortetracycline	>100
Oxytetracycline	41
Doxycycline	25

NOTES:

CUSTOMER SERVICE

Neogen Customer Assistance and Technical Services can be reached by using the contact information on the back of this booklet. Training on this product, and all Neogen test kits, is available.

SDS INFORMATION AVAILABLE

Safety data sheets (SDS) are available for this test kit, and all of Neogen's test kits, on Neogen's website at foodsafety.neogen.com, or by calling Neogen at 800/234-5333 or 517/372-9200.

TERMS AND CONDITIONS

For Neogen's full terms and conditions, please visit www.neogen.com/Corporate/termsconditions.html.

WARRANTY

Neogen makes no warranty of any kind, either expressed or implied, except that the materials from which its products are made are of standard quality. There is no warranty of merchantability of this product, or of the fitness of the product for any purpose. Neogen shall not be liable for any damages, including special or consequential damage, or expense arising directly or indirectly from the use of this product.

OTHER RESIDUE DIAGNOSTICS KITS

RT-96-TT-AMS	**ALERT for Ractopamine** — qualitative microwell assay, 96 wells
9551	**Veratox for Chloramphenicol** — range 10–1,000 ppt, 96 wells
8416	**Veratox for Malachite Green** — range 1–4 ppb, 48 wells
DR021	**Veratox for Clenbuterol** — range 0.08–1.28 ppb, 96 wells
DR107	**Veratox for Florfenicol** — range 0.15–100 ppm, 96 wells
DR073	**Veratox for Avermectins** — range 6.4–300 ppb, 96 wells

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