Tetracyclines ELISA Kit (OKAO00129)

Lot# KF0516

Instruction for Use

For the quantitative detection of Tetracyclines concentration in tissue, liver, egg, honey and urine.

Variation between lots can occur. Refer to the manual provided with the kit.

This product is intended for research use only.

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1. Background

Principle

Aviva Systems Biology Tetracyclines ELISA Kit (OKAO00129) is based on standard competitive inhibition enzyme-linked immuno-sorbent assay technology. Tetracyclines has been pre-coated onto a 96-wellplate (12 x 8 Well Strips). Standards or test samples are added to the wells along with a Tetracyclines Detector antibody and an HRP Conjugated antibody, then incubated. Wells are washed and detection substrates are added and incubated. An enzymatic reaction is produced through the addition of TMB which is catalyzed by HRP generating a blue color product that changes yellow after adding acidic stop solution. The density of yellow coloration read by absorbance at 450 nm which is quantitatively proportional to the amount of detector antibody bound in the well and inversely proportional to the amount of Tetracyclines in the sample.

Background

Tetracyclines are a group of broad-spectrum antibiotics whose general usefulness has been reduced with the onset of Antibiotic resistance. Despite this, they remain the treatment of choice for some specific indications. They are so named for their four (“tetra-”) hydrocarbon rings (“-cycl-”) derivation (“-ine”). To be specific, they are defined as ""a subclass of polyketides having an octahydrotetracene-2-carboxamide skeleton"". They are collectively known as ""derivatives of polycyclic naphthacene carboxamide"". Tetracyclines are generally used in the treatment of infections of the urinary tract, respiratory tract, and the intestines and are also used in the treatment of chlamydia, especially in patients allergic to β-lactams and macrolides; however, their use for these indications is less popular than it once was due to widespread development of resistance in the causative organisms. Their most common current use is in the treatment of moderately severe acne and rosacea (tetracycline, oxytetracycline, doxycycline or minocycline). Doxycycline is also used as a prophylactic treatment for infection by Bacillus anthracis (anthrax) and is effective against Yersiniapestis, the infectious agent of bubonic plague. It is also used for malariatreatment and prophylaxis, as well as treating. elephantiasis. Tetracyclines remain the treatment of choice for infections caused by chlamydia (trachoma, psittacosis, salpingitis, urethritis and L. venereum infection), Rickettsia (typhus, Rocky Mountain spotted fever), brucellosis and spirochetal infections (borreliosis, syphilis and Lyme disease). In addition, they may be used to treat anthrax, plague, tularemia and Legionnaires ‘disease. They are also used in veterinary medicine. They may have a role in reducing the duration and severity of cholera, although drug-resistance is mounting and their effect on overall mortality is questioned. Tetracycline derivatives are currently being investigated for the treatment of certain inflammatory disorders.

General Specifications

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Range	0.05 – 4.05 ppb
LOD	< 0.05 ppb (Derived by linear regression of OD_{450} of the Mean Blank + 2xSD)
Specificity	Tetracyclines CAS#: 60-54-8 PubChem#: 54675776 Alias: TET; Tetracycline; Deschlorobiomycin; Tetracyclinum; Achromycin; 60-54-8; Tetracyclin

2. Storage and Stability

• Upon receipt store kit at 4°C. Do not use past expiration date.

3. Kit Components

•The following reagents are the provided contents of the kit.

Description	Quantity	Storage Conditions
Tetracyclines Microplate	96 Wells (12 x 8 Well strips)	Store at 4°C Do not use past expiration date
Tetracyclines High Concentration Standard	1 x 1 mL	
Tetracyclines Standards	6 x 0 mL	
Tetracyclines Detector Antibody	1 x 6 mL	
HRP Conjugate	2 x 6 mL	
Detection Reagent A	1 x 6 mL	
Detection Reagent B	1 x 6 mL	
5X Sample Prep Buffer	2 x 20 mL	
20X Wash Buffer	1 x 20 mL	
Stop Solution	1 x 6 mL	

4. Required Materials Not Supplied

• Microplate reader capable of reading absorbance at 450 nm.
• Automated plate washer (optional).
• Pipettes capable of precisely dispensing 0.5 µL through 1 mL volumes of aqueous solutions.
• Pipettes or volumetric glassware capable of precisely measuring 1 mL through 100 mL of aqueous solutions.
• New, clean tubes and/or micro-centrifuge tubes for the preparation of standards or samples.
• Absorbent paper or paper toweling.
• Distilled or deionized ultrapure water.
• Heater water bath
• Centrifuge
• Reagents: N,N-Dimethylformamide (DMF)

5. Precautions

• Read instructions fully prior to beginning use of the assay kit.

• Any deviations or modifications from the described method or use of other reagents could result in a reduction of performance.

• Reduce exposure to potentially harmful substances by wearing personal protective lab equipment including lab coats, gloves and glasses.

• For information on hazardous substances included in the kit please refer to the Material Safety Data Sheet (MSDS).

• Kit cannot be used beyond the expiration date on the label.

6. Technical Application Tips

• Do not mix or substitute components from other kits.

• To ensure the validity of experimental operation, it is recommended that pilot experiments using standards and a small selection of sample dilutions to ensure optimal dilution range for quantitation.

• Samples exhibiting OD measurements higher than the highest standard should be diluted further in the appropriate sample dilution buffers.

• Prior to using the kit, briefly spin component tubes to collect all reagents at the bottom.

• Replicate wells are recommended for standards and samples.

• Cover microplate while incubating to prevent evaporation.

• Do not allow the microplate wells dry at any point during the assay procedure.

• Do not reuse tips or tube to prevent cross contamination.

• Avoid causing bubbles or foaming when pipetting, mixing or reconstituting.

• Completely remove of all liquids when washing to prevent cross contamination.

• Prepare reagents immediately prior to use and do not store, with the exception of the top standard.

• Equilibrate all materials to ambient room temperature prior to use (standards exception).

• For optimal results for inter- and intra-assay consistency, equilibrate all materials to room temperature prior to performing assay (standards exception) and perform all incubations at 37°C.

• Pipetting less than 1 µL is not recommended for optimal assay accuracy.

• Once the procedure has been started, all steps should be completed without interruption. Ensure that all reagents, materials and devices are ready at the appropriate time.

• Incubation times will affect results. All wells should be handled in the same sequential order and time intervals for optimal results.

• Samples containing precipitates, fibrin strands or bilirubin, or are hemolytic or lipemic might cause inaccurate results due to interfering factors.

• TMB Substrate is easily contaminated and should be colorless or light blue until added to plate. Handle carefully and protect from light.

• To minimize influences on the assay performance, operation procedures and lab conditions, especially room temperature, air humidity, incubator temperature should be strictly controlled. It is also strongly suggested that the whole assay is performed by the same operator from the beginning to the end.

7. Reagent Preparation

• Equilibrate all materials to room temperature prior to use and use immediately.

• The following reagents are provided at ready to use concentrations and require no preparation:

- Tetracyclines High Concentration Standard
- Tetracyclines Detector Antibody
- HRP Conjugate
- Detection Reagent A
- Detection Reagent B

7.1 1X Wash Buffer

7.1.1 Add the 20 mL of **20X Wash Buffer** to 380 mL of ultrapure water.

7.1.2 Mix gently and thoroughly. Store at 4°C for one week.

7.2 1X Sample Prep Buffer

7.2.1 Add the 20 mL of **5X Sample Prep Buffer** to 80 mL of ultrapure water.

7.2.2 Mix gently and thoroughly. Store at 4°C for one week.

7.3 Standards

The standard working solution needs to be prepared before the experiment. Low concentration standards are unstable and need to be used right after prepared.

7.3.1 Prepare the set of standards as follows:

7.3.1.1 Use **Tetracyclines High Concentration Standard** as the high standard point.

7.3.1.2 Add 2 mL **1X Sample Prep Buffer** to Standard #'s 1 – 4.

7.3.1.3 Add 3 mL **1X Sample Prep Buffer** to Standard # 5.

7.3.1.4 Prepare **Standard # 5** by adding 12 µL **High Concentration Standard**.

7.3.1.5 Prepare **Standard #4** by adding 1 mL of **Standard # 5** from **Vial #5** to **Vial # 4**. Mix gently and thoroughly.

7.3.1.6 Prepare further dilutions through **Vial #1**. Reference the table below as a guide for dilution scheme.

7.3.1.7 **Standard #0** is a blank standard (only 1 mL **1X Sample Prep Buffer**).

Standard Number (Vial)	Standard To Dilute	Volume Standard to Dilute (µL)	Volume 1X Sample Prep Buffer (µL)	Total Volume (µL)	Final Concentration
High Concentration Standard	1.0 ppm	1000	NA	1000	1.0 ppm
5	1.0 ppm	12	3000	3012	4.05 ppb
4	4.05 ppb	1000	2000	3000	1.35 ppb
3	1.35 ppb	1000	2000	3000	0.45 ppb
2	0.45 ppb	1000	2000	3000	0.15 ppb
1	0.15 ppb	1000	2000	3000	0.05 ppb
0	NA	NA	1000	1000	0 ppb

7.4 Microplate Preparation

• Equilibrate all reagents and materials to ambient room temperature prior to use in the procedure.
• Temperature regulation for all procedures and incubations to 25°C is recommended for optimal reproducibility.

8. Sample Preparation

8.1 Sample Preparation and Storage

• Mix diluted samples gently and thoroughly.
• Pipetting less than 2 µL is not recommended for optimal assay accuracy.
• Untreated samples should be stored at -20°C.
• Prepared samples can be stable for 1 week at 4°C.

8.1.1 Tissue, liver, egg: (Dilution 1:8)

8.1.1.1.1 Homogenize the sample.
8.1.1.1.2 Weigh 2.0 ± 0.05 g of the homogenized sample into a 50 mL centrifugal tube, add 4 mL **DMF.**
8.1.1.1.3 Vortex for 5 minutes.
8.1.1.1.4 Centrifuge at above 4,000 rpm at room temperature (20-25 °C) for 10 minutes.
8.1.1.1.5 Transfer 250 µL sample solution into a 5 mL centrifuge tube.
8.1.1.1.6 Add 750 µL of **1X Sample Prep Buffer**, mix properly for 30 seconds.
8.1.1.1.7 Take 50 µL for further analysis.

8.1.2 Honey (Dilution 1:40)

8.1.2.1 Weigh 1.0±0.05 g honey into 10 mL centrifugal tube, then add 2 mL **DMF**. Vortex for 2 minutes to dissolve completely.
8.1.2.2 Centrifuge at greater than 4,000 rpm at room temperature (20-25°C) for 10 minutes.
8.1.2.3 Transfer 100 µL of the supernatant into a 5 mL centrifuge tube.
8.1.2.4 Add 1.9 mL of the **1X Sample Prep Buffer** and vortex for 30 seconds.
8.1.2.5 Take 50 µL for further analysis.

8.1.3 Urine (Dilution 1:10)

8.1.3.1 Measure 100 µL clear urine. Store in frozen environment if not used.
8.1.3.2 If urine is muddy, centrifuge at 4,000 rpm at room temperature (20-25°C) for 10 minutes, then measure clear urine.
8.1.3.3 Add 900 µL of the **1X Sample Prep Buffer** and vortex for 2 minutes
8.1.3.4 Centrifuge at 4000 rpm at room temperature for 20-25°C for 5 minutes.
8.1.3.5 Take 50 µL of clear supernatant for further analysis.

9. Assay Procedure

• Equilibrate all reagents and materials to ambient room temperature prior to use in the procedure.

• Temperature regulation for all procedures and incubations to 25°C is recommended for optimal reproducibility.

9.1 Determine the required number of wells and return any remaining unused wells and desiccant to the pouch.

9.2 Add 50 µL of standards or samples into wells of the **Tetracyclines Microplate**. At least two replicates of each standard, sample or blank is recommended.

9.3 Immediately add 50 µL of the **Tetracyclines Detector Antibody** to each well.

9.4 Cover the plate with the plate sealer, gently mix and incubate for 30 minutes.

9.5 Remove the plate sealer and discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle or aspiration.

9.6 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

9.7 Wash plate 5 times with **1X Wash Buffer** as follows:

9.7.1 Add 250 µL of **1X Wash Buffer** to each assay well.

9.7.2 Incubate for 30 seconds.

9.7.3 Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle.

9.7.4 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

9.7.5 Repeat steps 9.7.1 through 9.7.4 **four** more times.

9.8 Add 100 µL of the **HRP Conjugate** to each well.

9.9 Cover the plate with the plate sealer, gently mix and incubate for 30 minutes.

9.10 Remove the plate sealer and discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle or aspiration.

9.11 Repeat washing process in step 9.7.

9.12 Add 50 µL of prepared **Detection Reagent A** and 50 µL of **Detection Reagent B** to each well.

9.13 Gently mix the plate and incubate for 15 minutes **in the dark**.

9.14 Add 50 µL of **Stop Solution** to each well and mix gently. Well color should change to yellow immediately. Add the **Stop Solution** in the same well order as done for the **Detection Reagents**.

9.15 Read the O.D. absorbance at 450 nm with a standard microplate reader within 5 minutes of stopping the reaction in step 9.14. If wavelength correction is available, set to 540 nm or 570 nm.

10. Calculation of Results

For analysis of the assay results, first derive the **Percent Relative Absorbance** for each sample or standard by calculating the mean absorbance between replicate wells. This value is then divided by the mean replicate Blank (0 ppb) absorbance, then multiplying by 100%:

$$\text{Percent Relative Absorbance (Sample or Standard)} = \frac{\text{Mean Absorbance (Sample or Standard)}}{\text{Mean Blank Absorbance}} \text{ X } 100\%$$

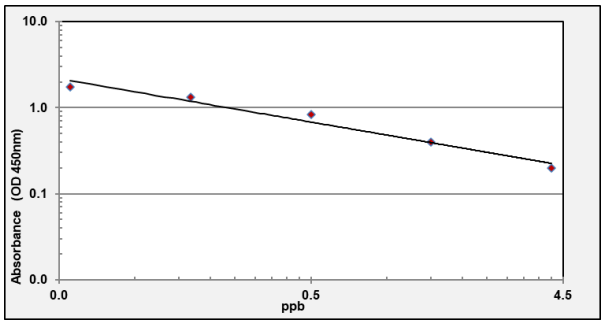
A standard curve is generated by plotting the **Percent Relative Absorbance** of each standard serial dilution point vs. the respective standard concentration. The Tetracyclines concentration contained in the samples can be interpolated by using linear regression of each sample **Percent Relative Absorbance** against the standard curve. This is best achieved using curve fitting software.

Note: If wavelength correction readings were available, subtract the readings at 540 nm or 570 nm from the readings at 450 nm. This may provide greater reading accuracy.

Note: If the samples measured were diluted, multiply the derived mean sample concentration by the dilution factor for a final sample concentration.

11. Typical Expected Data

11.1 Typical Data



ppb	Absorbance		Mean Absorbance	B/B0 (%)
	Rep 1	Rep 2		
4.05	0.197	0.204	0.201	9.4
1.35	0.397	0.386	0.392	18.3
0.45	0.828	0.809	0.819	38.4
0.15	1.313	1.325	1.319	61.9
0.05	1.733	1.743	1.738	81.5
0.00	2.130	2.133	2.132	100.0

11.2 Limit of Detection

Derived by linear regression of OD450 of the Mean Blank + 2xSD

Sample	Mean Recovery
Tissue, Liver, Egg	0.4 ppb
Honey	2 ppb
Urine	0.5 ppb

11.3 Reproducibility

Three samples of known concentrations were measured in 20 replicates within an assay plate to asses intra-assay reproducibility:

Mean Intra-Assay Precision - ≤8% (n = 3 x 20)

Three samples of known concentrations were measured across 40 replicate assays inter-assay reproducibility:

Mean Inter-Assay Precision - ≤10% (n= 3 x 40)

Sample	Intra-Assay			Inter-Assay		
	1	2	3	1	2	3
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (ppb)	0.313	0.941	1.380	0.291	1.012	1.537
SD	0.019	0.063	0.099	0.020	0.075	0.124
CV (%)	6.1	6.7	7.2	6.9	7.4	8.1

11.4 Cross-Reactivity Rate

Substance	Cross Reactivity Rate
Tetracycline	100%
Oxytetracycline	107%
Aureomycin	16.7%
Medomycin	4.2%

11.5 Recovery

The recovery of Tetracyclines spiked at levels throughout the range of the assay in various matrices was evaluated. Samples were diluted prior to assay as directed in the Sample Preparation section.

Sample Type	Number	Recovery Range (%)
Tissue, Liver, Egg	10	85 ± 20
Honey	10	75 ± 20
Urine	10	80 ± 20

12. Technical Resources

Technical Support:

For optimal service please be prepared to supply the lot number of the kit used.

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