Tetracyclines ELISA Kit

rev 01/19

(Catalog # E4273-100, 100 assays, Store at 4°C)

I. Introduction:

Tetracycline is an antibiotic used to treat a number of bacterial infections. It is commonly used to treat acne and rosacea. Historically it was important in reducing the number of deaths from cholera. A broad-spectrum antibiotic of the polyketide class, it is produced by the actinobacterial genus Streptomyces. It acts by inhibiting protein synthesis. It is first-line therapy for rocky mountain spotted fever, Lyme disease, Q fever, psittacosis and lymphogranuloma venereum, mycoplasma pneumoniae and to eradicate nasal carriage of meningococci. BioVision's Tetracycline ELISA kit is a competitive ELISA assay for the quantitative measurement of Tetracycline in tissues, honey and urine. The density of color is proportional to the amount of tetracycline captured from the samples.

II. Application:

This ELISA kit is used for *in vitro* quantitative determination of Tetracycline.
Detection Range: 0.5 – 40 ppb (ng/ml)
Sensitivity: < 0.05 ppb
Detection limit: 0.4 ppb for tissue, liver egg; 0.5 ppb for urine, 2 ppb for honey
Cross Reactivity: Tetracyclines: 100%, Chlortetracycline: 16.7%, Oxytetracycline: 107%, Doxycycline: 4.2%

III. Sample Type:

Tissue, honey, liver, egg

IV. Kit Contents:

Components	E4273-100	Part No.
Micro ELISA Plate	8 X 12 strips	E4273-100-1
Plate Sealer	1	E4273-100-2
High standard (1000 ng/ml)	1.0 ml	E4273-100-3
Antibody working solution	5.5 ml	E4273-100-4
Enzyme conjugate	11 ml	E4273-100-5
Substrate A solution	6 ml	E4273-100-6
Substrate B solution	6 ml	E4273-100-7
Stop Solution	6 ml	E4273-100-8
Concentrated Wash Solution (20X)	40 ml	E4273-100-9
Concentrated Redissolving solution (5X)	50 ml	E4273-100-10

V. User Supplied Reagents and Equipment:

- Reagents: 1% solution of trichloroacetic acid, methanol
- Microplate reader capable of measuring absorbance at 450 nm
- Nitrogen-drying device
- Clean tubes for preparing standards or sample dilutions
- Absorbent paper

VI. Storage and Handling:

The entire kit may be stored at 4°C for up to 12 months from the date of shipment.

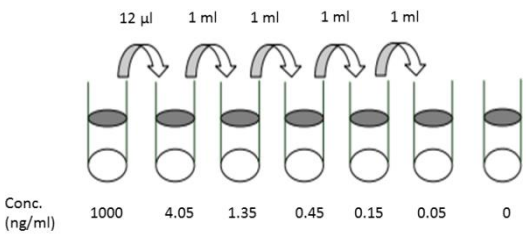
VII. Reagent Preparation:

Note: Prepare reagents within 30 minutes before the experiment.
Before using the kit, spin tubes and bring down all components to the bottom of tubes.

1. **Redissolving solution**: Dilute the concentrated redissolving solution 5 times with deionized water to be used for sample redissolving, it can be stored at 4 ℃ environment up to a month.
2. **Standards**: Prepare 6 new tubes. Pipette 3 ml redissovling solotion in the 1st and 6th tube. Pipette 2 ml redissolving solution in other tubes. Pipette 12 µl high standard in the 6th tube and mix well. Pipette 1 ml from the 6th tube to the 5th tube and conduct similar procedure (serial dilution) until 2nd tube. Use first tube as blank (0 ng/ml).
3. **Wash Buffer:** Dilute 40 ml of the concentrated washing buffer with the distilled or deionized water to 800 ml (or just to the required volume) for using.
4. **Sample Preparation**:

   Note: Samples to be used within 5 days may be stored at 4°C, otherwise samples must be stored at -20°C (≤1 month) or -80°C (≤2 months) to avoid loss of bioactivity and contamination. Avoid multiple freeze-thaw cycles.

   - **Tissue, liver, egg samples:** Weigh 2 g homogenized sample into 50 ml centrifuge tube, add 4 ml 1% solution of trichloroacetic acid, oscillate 2 min, centrifuge at 4000 rpm at room temperature for 10 min. Transfer 250 µl supernatant to another centrifuge tube and blow dry at 50 - 60°C with nitrogen



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or air. Add 750 µl redissolving solution to dissolve the dried residue, mix. Use 50µl for the assay. (Dilution times of the sample: 1:8)

- **Honey**: Weigh 1 g Honey sample into centrifuge tube, add 2ml 1% trichloroacetic acid and oscillate for 2 min, centrifuge at 4000 rpm at room temperature for 10 min. Wipe out 100µl supernatant to another centrifuge tube, Add 1900 µl redissolving solution to dilute, mix for 30 sec. Use 50 µl for the assay. (Dilution times of the sample: 1: 40)
- **Urine:** dilute the urine sample 5 times with redissolving solution (if the urine is turbid, filter or centrifuge at 4000 rpm at room temperature for 10 min), the unused sample should be kept frozen. Use 50µl diluted sample for the assay. (Dilution times of the sample: 1: 10)

VIII. Assay Protocol:

Note: Bring all reagents and samples to room temperature 30 minutes prior to the assay. Shake the reagent bottles if there is any crystal. It is recommended that all standards and samples be run at least in duplicate. A standard curve must be run with each assay.

1. Prepare all reagents, samples and standards as instructed in section VII.
2. Add 50 µl **diluted standards** or **samples** into marked well. Add 50 µl **antibody working solution** into each well.
3. Oscillate the plate for 5 sec, cover the well and incubate in dark for 30 min at 37°C.
4. Discard solution, wash plate 5 times with **1X Wash Solution**. Wash by filling each well with Wash Buffer (250 µl) using a multi-channel pipette or autowasher. Let it soak for 1 min, and then remove all residual wash-liquid from the wells. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Clap the plate on absorbent filter papers or other absorbent materials.
5. Add 100 µl **Enzyme conjugate** into each well; avoid the light to incubate for 30 min at 37°C.
6. Repeat washing procedure to step 4.
7. Pipette 50 µl **Substrate A solution**, then pipette 50 µl **Substrate B solution** to each well, oscillate gently for 5 sec, avoid the light preservation for 15 min at 37°C.
8. Add 50 µl **Stop Solution** to each well and oscillate gently to stop the reaction.
9. Read result at 450 nm within 10 minutes.

IX. CALCULATION:

Percentage of absorbance value (%) = A/A_0 X 100%

A: the average (double wells) OD value of the sample or the standard solution; A_0: the average OD value of the 0 ppb standard solution.

To draw the standard curve and calculate, take absorbance percentage of standards as Y-axis, the corresponding log of standards concentration (ppb) as X-axis. Draw the standard semilog curves with X-axis and Y-axis. Take absorbance percentage of samples substitute into standard curve, then can get the corresponding concentration from standard curve; last, Multiplied by the corresponding dilution times is the actual concentration of Sal of samples.

X. RELATED PRODUCTS:

- Salbutamol (SALB) ELISA Kit (Cat. No. K4209-100)
- Sulfonamides residue ELISA Kit (Cat. No. K4207-100)
- Aflatoxin B1 (AFB1) ELISA Kit (Cat. No. K4208-100)
- Fluoroquinolones ELISA Kit (Cat. No. K4205-100)
- Gentamicin ELISA Kit (Cat. No. K4206-100)
- Kanamycin ELISA Kit (Cat. No. K4210-100)
- Streptomycin ELISA Kit (Cat. No. E4272-100)
- Melamine ELISA Kit (Cat. No. E4274-100)