

## ab285261 – Tetracyclines ELISA Kit

For quantitative measurement of Tetracyclines in tissue, honey, liver and egg.  
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:

<http://www.abcam.com/ab285261>

### Storage and Stability

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

### Materials Supplied

Item	Quantity	Storage Condition
Micro ELISA Plate	1	4°C
High Standard (1000 ng/ml)	1 ml	4°C
Antibody Working Solution	5.5 ml	4°C
Enzyme Conjugate	11 ml	4°C
Substrate A solution	6 ml	4°C
Substrate B solution	6 ml	4°C
Stop Solution	6 ml	4°C
Concentrated Wash Solution (20X)	40 ml	4°C
Concentrated Redissolving Solution (5X)	50 ml	4°C
Plate sealer	1	4°C

### Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

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

### Reagent Preparation

**Δ 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.

**Redissolving solution:** Dilute the concentrated redissolving solution 5 times with deionized water to be used for sample redissolving, it can be stored at 4 °C for up to a month.

**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.

### Standard Preparation

- 1) Prepare 6 new tubes.
- 2) Pipette 3 ml redissolving solution in the 1st and 6th tube.
- 3) Pipette 2 ml redissolving solution in other tubes.
- 4) Pipette 12 µl high standard in the 6th tube and mix well.
- 5) Pipette 1 ml from the 6th tube to the 5th tube and conduct similar procedure (serial dilution) until 2nd tube.
- 6) Use first tube as blank (0 ng/ml).

### Sample Preparation

**Δ 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 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)

### Assay Protocol

**Δ Note:** Bring all reagents and samples to room temperature 30 minutes prior to the assay. 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
- 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.

### Measurement

Read absorbance at 450 nm within 10 minutes of adding stop solution.

### Calculation:

$$\text{Percentage of absorbance value (\%)} = A/A_0 \times 100\%$$

A: the average (double wells) OD value of the sample or the standard solution  
A<sub>0</sub>: 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 semi log curves with X-axis and Y-axis.

- Plot the absorbance percentage of samples into the standard curve, to get the concentration for each sample and multiply by the dilution to give the concentration of streptomycin in samples.

### **Technical Support**

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