Streptomycin ELISA Kit Manual

(Art. No.: KITW003.01)

【Product Description】

The kit (Art. No.: KITW003.01) is an enzyme-linked immunosorbent assay (ELISA) for the quantitative determination of Streptomycin in honey and milk.The specificity of the kit has been evaluated by analyzing the cross-reactivities to corresponding substances. The kit is a convenient, fast, and sensitive assay for on-site large-scale screening of Streptomycin in honey and milk.

【Technical Specifications】

1. **Sensitivity**: 0.2 ppb.
2. **Detection Limit**: 15 ppb, milk and honey
3. **Recovery rate**: 80%-110%, milk and honey
4. **Specificity**:
   Streptomycin----------100%
   Dihydrostreptomycin ----------106%
5. **Precision**： CV < 10%

【Components provided】

No.	Component	Quantity
1	Standards： 0 ppb 0.2 ppb 0.4 ppb 1.2 ppb 3.6 ppb 10.8 ppb	 1 vial (1.5 mL) 1 vial (1.5 mL) 1 vial (1.5mL) 1 vial (1.5 mL) 1 vial (1.5 mL) 1 vial (1.5 mL)
2	Microtiter plate	1 x 96 well plate (8 wells x 12 strips)
3	Antibody solution	1 bottle (7 mL)
4	Enzyme conjugate solution	1 bottle (12 mL)
5	Sample dilution concentrate (5×)	1 bottle (25 mL)
6	Washing solution concentrate (20×)	1 bottle (25 mL)
7	Substrate A solution	1 bottle (7 mL)
8	Substrate B solution	1 bottle (7 mL)

9	Stop solution	1 bottle (7 mL)
10	Adhesive plate sealer	1 pc
11	Self-sealing bag	1 pc
12	Instruction manual	1 book

【Storage instructions】

1. Store the kit/reagents at 2-8℃. The shelf life is 12 months when properly stored.
2. Return unused micro-wells to their original foil bag and reseal them together with provided desiccant and further store at 2-8℃.

【Materials Required but not Provided】

1. Equipment:

a. Microtiter plate reader (450 nm)
b. Precision balance (0.01 g)
c. Vortex
d. Centrifuge (4000 g)
e. Graduated micropipette/ tips
f. Timer

2. Reagent:

a. 1% trichloroacetic acid (Mix 1 g trichloroacetic acid with 99 mL of distilled water)
b. Distilled water

【Reagent Preparation】

1. **Wash Solution**: Mix 1 volume of **Wash Solution Concentrate (20×)** with 19 volumes of distilled water.
2. **Sample dilution**: Mix 1 volume of **Sample dilution concentrate (5×)** with 4 volumes of distilled water.
3. **Sample dilution 2#**:Mix 1 volume of **Wash Solution Concentrate (20×)** with 9 volumes of distilled water.
4. **TMB substrate Solution:** Mix **Substrate A Solution** with equal volume of **Substrate B Solution**. Use it within 5 min.(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.)

【Sample Preparation】

Make sure samples are properly stored.

Honey (Dilution factor: 10)

1. Transfer 1±0.05 g sample to 10 mL centrifuge tube.
2. Add 4 mL distilled water , then mix vigorously (vortex) to dissolve the honey completely.
3. Transfer 200 µL solution Solution into a new 2 mL centrifuge tube.
4. Add 200 µL **sample dilution 2#**, mix thoroughly for 30 s.
5. Pipette the aqueous layer for assay (with no further dilutions needed).

Milk (Dilution factor: 40)

1. Transfer 1±0.05 g sample to 10 mL centrifuge tube.
2. Add 1 mL **1% trichloroacetic acid** , then mix vigorously (vortex) for 1 min.
3. Centrifuge at 4000 g for 5 min at room temperature.
4. Transfer 50 µL middle-solution into a new 2 mL centrifuge tube.
5. Add 950 µL **sample dilution**, mix thoroughly for 30 s.
6. Pipette the aqueous layer for assay (with no further dilutions needed).

【Test Procedure】

1. Insert sufficient number of wells into a micro well holder. Record standard and sample positions. It is suggested that all standards and samples be run in duplicate.
2. Add 50 µL of each standard or sample into separate duplicate wells.
3. Add 50 µL of **Antibody solution** to each well.
4. Cover it with **Adhesive plate sealer**, and mix gently by shaking for 10 seconds. Incubate for 30 min at room temperature(25±2℃) in the dark.
5. Uncover the adhesive plate sealer and discard the liquid in wells.
6. Wash the plate 4 times with 260 µL of washing solution.
7. After the last wash, tap dry the plate on paper towel.
8. Add 100 µL of **Enzyme conjugate solution** to each well.
9. Cover it with **Adhesive plate sealer**, and mix gently by shaking for 10 seconds. Incubate for 30 min at room temperature(25±2℃) in dark place.
10. Repeat step 5-7.
11. Add 100 µL of **TMB substrate solution** into each well.
12. Repeat step 4 but incubate for 15-20 min.
13. Uncover the **adhesive plate sealer** and add 50 µL of **stop solution** into each well. Mix gently by shaking 10 seconds.

14. Measure the absorbance with **Microtiter plate reader** at 450 nm within 5 min.

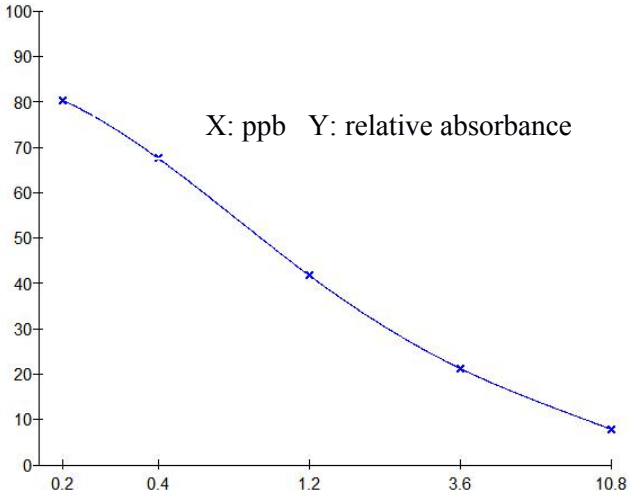
【Results interpretation 】

1. Using the OD values (relative absorbance) expressed as a percentage of the OD of the zero (standard), construct a dose-response curve using the five standards. Since the analyte in each standard is known, the unknowns in the sample can be measured by interpolation from the standard curve.

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

2. To obtain the Streptomycin concentration actually contained in a sample, the concentration read from the standard curve must be further multiplied by the corresponding dilution factor. The following is a typical Streptomycin standard curve of the kit.

Streptomycin Standard Curve



Standard (ppb)	0	0.2	0.4	1.2	3.6	10.8
Relative absorbance	2.168	1.744	1.466	0.906	0.461	0.170

3. This kit is only for screening test, positive result should be further confirmed with other method(HPLC, LC, MS).

【Notes】

1. Please read the manual carefully before assay.
2. Do not use the kit past expiration date.
3. Do not intermix micro-wells and reagents from different kits or lots.
4. All reagents should be restore up to room temperature before use (1-2 hours at 20-25℃).
5. Incubation times and temperatures of assay should be controlled as precisely as possible. Be

6. consistent when adding standards and samples.
7. To avid time lag, please use 8-channel pipette when adding Enzyme Conjugate Solution, Wash Solution, and the like.
8. Please make sure the samples are fresh or properly stored. In general, samples should be refrigerated at 2-4℃ for no more than 1-2days.
9. Any metallic substance should be avoided to contain or agitate reagents.
10. Shake each reagent well before use especially for solutions with crystal liberation. Avoid air bubbles when mixing.
11. Avoid running assays under or near air vents, as this may cause excessive cooling, heating and/or evaporation.
12. The stop solution contains strong acid substances. Please wash by water immediately if it splashes on clothes or skin.
13. Please do not reuse pipette tips.
14. All wastes should be properly decontaminated prior to disposal. Dispose of contents in accordance with local, regional, and national regulations.

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