Elabscience®

7th Edition, revised in April, 2017

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SM(Streptomycin) ELISA Kit

Catalog No: E-FS-E031

96T

This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help.

Phone: 240-252-7368(USA)240-252-7376(USA)
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Test principle

This kit uses Indirect-Competitive-ELISA as the method. It can detect Streptomycin (SM) in tissues, honey and other samples. This kit is composed of Micro ELISA Plate pre-coated with coupled antigen, HRP conjugate, antibody, standard and other supplementary reagents. During the detection, after adding standard or sample solution, SM in the samples competes with pre-coated coupled antigen on the Micro ELISA Plate for SMantibody.Then Horseradish Peroxidase (HRP) conjugate is added to each micro plate well, and TMB substrate is added for color development. There is a negative correlation between the OD value of samples and the concentration of SM. The residual quantity of SM in the samples can be calculated by comparing the OD of the samples to the standard curve.

Technical indicator

Sensitivity:0.1 ppb(ng/mL)

Reaction mode:25℃, 30min~30min~ 15min

Detection limit: Tissue ---4 ppb; Honey---2 ppb; Milk,milk powder ---5 ppb.

Cross-reactivity: Streptomycin---100%,Dihydrostreptomycin---100%,Kalamycin---6.3%, Gentamicin ---2.5%.

Sample recovery rate: Tissue, Honey, Milk---85%±15%.

Kits components

Item	Specifications
Micro ELISA Plate	96 wells
Standard Solution	1mL each (0ppb,0.1ppb,0.3ppb,0.9ppb, 2.7 ppb, 8.1ppb)
High Concentrated Standard (1ppm)	1mL
HRP Conjugate (Red lid)	11mL
Antibody Working Solution(Blue lid)	5.5mL
Substrate Reagent A(White lid)	6mL
Substrate Reagent B(Black lid)	6mL
Stop Solution(Yellow lid)	6mL
20×Concentrated Wash Buffer(White lid)	40mL
5× Re-dissolve solution(Yellow lid)	50mL
Product Description	1 copy

Other supplies required

Instruments: Microplatereader, Printer, Homogenizer, Nitrogen Evaporators, Oscillators, Centrifuge, Graduated pipette, Balance (sensibility 0.01g).

Micropipettor: Single-channel (20-200μL, 100-1000μL), Multi-channel (300μL).

Reagents: NaOH,$Na_2HPO_4 \cdot 12H_2O$,$NaH_2PO_4 \cdot 2H_2O$,N-hexane,Dichloromethane,Acetonitrile,Phosphoric acid.

Experimental preparation

Bring all reagents and samples to room temperature before use.

Open the micro-plate reader in advance, preheat the instrument, and set the testing parameters.

1. **Sample pretreatment Notice:** experimental apparatus should be clean, and the pipette should be disposable to avoid cross-contamination during the experiment.

2. **Solution preparation**

Solution 1:0.05MPB buffer

Dissolve 12.9 g of $Na_2HPO_4 \cdot 12H_2O$and 2.175 g $NaH_2PO_4 \cdot 2H_2O$with1000mL deionized water.

Solution 2:0.04M Phosphoric acid (for honey sample)

Dilute 1 mLconcentrated phosphoric acid with deionized water to 360 mL.

Solution 3:1M NaOH (for honey sample)

Dissolve 4 g NaOH with deionized water to make 100 mL.

Solution 4: Re-dissolve solution

Dilute the 5×Re-dissolvesolution to 1 ×Re-dissolve solutionwith deionized water for re-dissolution of samples. The re-dissolve solution is stable for 1 month at 4℃.

3. **Sample pretreatmentprocedure**

3.1 Pretreatment oftissue:

(1) Weigh 2±0.05g of homogenates ample without fat, add 8mL of 0.05M PB buffer. Oscillate for 5min and incubate at 56℃ water-bath for 30 min;

(2) Centrifuge at a speed of over 4000r/min for 10min at room temperature;

(3) Take 1 mLof the upper liquid and add 1 mLn-hexane. Mix fully and centrifuge at a speed of over 4000r/min for 5 min at room temperature;

(4) Remove the upper organic phase, take 50 μL of the lower liquid. Add 450μL of 1×Re-dissolve solution, mix for 30 s;

(5) Take 50 μL for analysis.

Note: Sample dilution factor: 40, minimum detection dose: 4ppb.

3.2 Pretreatment ofhoney and royal jelly:

(1) Weigh 2±0.05g ofhoney,add 4mL of0.04 M phosphoric acid, oscillate until it dissolved fully. Centrifuge at a speed of over 4000r/min for 10min at room temperature until the solution became

clear. (Honey sample can be processed step 2 without centrifugation.)

(2) Add 450 μLof 1M NaOH to adjust the pH to 7-9 (transfer the supernatant of royal jelly sample to another centrifuge tube and adjust the pH to 7-9). Centrifuge at a speed of over 4000r/min for 10min at room temperature until the solution became clear.

(3) Take 50 μL of supernatant and add 450μL of 1×Re-dissolve solution, mix for 30 seconds.

(4) Take 50 μL for analysis.

Note: Sample dilution factor: 20, minimum detection dose: 2 ppb.

3.3 Pretreatment ofmilk and milk powder:

(1) Weigh 2±0.05 g of sample, add 8 mL of 0.05M PB buffer, oscillate for 5 min, incubate at 56℃ water-bath for 30 min.

(2) Centrifuge at a speed of over 4000r/min for 10min at room temperature.

(3) Remove the upper layer fat and take 50 μL of the middle layer clear liquid. Add 450μL of diluted 1×Re-dissolve solution, mix for 30 s.

(4) Take 50 μL for analysis.

Note: Sample dilution factor: 50, minimum detection dose: 5 ppb.

Assay procedure

Bring all reagents and samples to room temperature for more than 30 min before use. The washing solution may be crystallized when refrigerated, therefore it should be adjusted to room temperature to fully dissolve. Each liquid must be shaken up before use. Take out the Micro plate and frame of needed amount, and keep the remained Micro plate into the valve bag and stored at 2-8℃.

Dilute the 20×Concentrated wash buffer to wash working buffer before the experiment.

1. **Number:**Number the sample and standard in order (multiple well), and keep a record of standard wells and sample wells.
2. **Add sample:**Add50μL of Standard or Sample per well, then add 50μL antibody working solution, cover the plate with sealer provided in the kit. Oscillate for 5s gently to mix thoroughly, shading light incubation for 30min at25℃.
3. **Wash:**Uncover the sealer carefully, remove the liquid in each well. Immediately add 250μLof wash working buffer to each well and wash.Repeat wash procedure for 5 times, 30s intervals/time. Invert the plate and pat it against thick clean absorbent paper (If bubbles exist in the wells, clean tips can be used to prick them).
4. **HRP conjugate:** Add 100μL HRP conjugate to each well, incubate for 30min at 25℃in the dark.
5. **Wash:**Repeat step 3.
6. **Color Development:** Add 50μLof substrate solution A to each well, and then add 50μLof substrate solution B.Gently oscillate for 5s to mix thoroughly. Incubate shading light for 15min at 25℃.
7. **Stop reaction:**Add 50μLof stop solution to each well, gently oscillate and mix fully to stop the reaction.
8. **OD Measurement:**Determine the optical density (OD value) of each wellat 450 nm with a microplate reader (the 450/630 nm double wavelength is recommended). This step should be

finished in 10min after stop reaction.

Result analysis

1. **Absorbance% =A/A_0×100%**

   A: Average absorbance of standard solution or sample

   A_0: Average absorbance of 0 ppb Standard solution

2. **Drawing and calculation of standard curve**

   Create a standard curve by plotting the absorbance percentage of each standard on the y-axis against the log concentration on the x-axis to draw a semi-logarithmic plot. Add the average absorbance value to standard curve to get corresponding concentration. If samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor.

   For this kit, it is more convenient to use professional analysis software for accurate and fast analysis on a large number of samples.

Notes

1. Overall OD value will be lower when reagents have notbeen brought to room temperature before use or room temperature ＜25℃.
2. During the washing procedure, if the wells turn dry, it will lead to bad linear standard curveand poor repeatability, move on to the next step immediately after wash.
3. Mix thoroughly and wash the plate completely. The consistency of wash procedure can strongly affect the reproducibility of this ELISA kit.
4. Micro ELISA plate should be covered by plate sealer. Avoid the reagents to strong light.
5. Do not use expired kit and reagents of different batches.
6. TMB should be abandoned if it turns color. When OD value of standard(concentration: 0)＜0.5 unit(A450nm＜0.5), it indicates the reagent may be deteriorated.
7. Stop solution is caustic, avoid contact with skin and eyes.

Storage and valid period

Storage: Store at 2-8℃. Avoid freeze / thaw cycles.

Valid Period: 1 year, production date is on the packing box.