SAR (Sarafloxacin) ELISA Kit

Catalog No: E-FS-E022

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)   Fax: 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.

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Test principle

This kit uses Indirect-Competitive-ELISA as the method. It can detect SAR in samples, such as honey, animal tissue (chicken, pork, fish, and shrimp), milk powder, egg, etc. This kit is composed of ELISA Microtiter plate, HRP conjugate, antibody, standard and other supplementary reagents. The microplate provided in this kit has been pre-coated with SAR. During the reaction, SAR in the samples or standard competes with SAR on the solid phase supporter for sites of SAR antibody. Then Horseradish Peroxidase (HRP) conjugate is added to each micro plate well, and TMB substrate is for color development. There is a negative correlation between the OD value of samples and the concentration of SAR. The concentration of SAR 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℃, 45 min～15 min

Detection limit: Tissue (chicken, pork, fish, shrimp) ---0.3 ppb, Honey ---0.4 ppb,
Milk ---3 ppb, Milk powder---6 ppb, Egg ---- 3 ppb, Urine --- 0.5 ppb

Cross-reactivity: Sarafloxacin ---100%,

Sample recovery rate: Tissue, Honey, Milk, Milk powder, Egg, Urine ---85%±15%

Kits components

Item	Specifications
ELISA Microtiter plate	96 wells
Standard Liquid	1 mL each (0ppb, 0.1ppb, 0.3ppb, 0.9ppb, 2.7ppb, 8.1ppb)
High Concentrated Standard (100ppb)	1 mL
HRP Conjugate	5.5 mL
Antibody Working Solution	5.5 mL
Substrate Reagent A	6 mL
Substrate Reagent B	6 mL
Stop Solution	6 mL
20×Concentrated Wash Buffer	40 mL
5×Reconstitution Buffer	50 mL
Plate Sealer	3 pieces
Sealed Bag	1 piece
Manual	1 copy

Note: All reagent bottle caps must be tightened to prevent evaporation and microbial pollution.

Other supplies required

Instruments: Microplate reader, Printer, Homogenizer, Nitrogen evaporators/Water bath, Oscillators, Centrifuge, Graduated pipette, Balance (sensibility 0.01 g).

High-precision transferpettor: single channel (20-200 μL, 100-1000 μL), Multichannel (300 μL).

Reagents: anhydrous acetonitrile、n-hexane、concentrated HCl.

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: 0.15 M HCl Solution

   Dissolve 5 mL of concentrated HCl to 400 mL with deionized water

   Solution 2: Sample Extract

   Measure 10 mL of 0.15 M HCl (Solution 1) to 90 mL of anhydrous acetonitrile, mix fully.

   Solution 3: Reconstitution Solution

   Dilute the 5×Reconstitution Buffer with deionized water. (5×Reconstitution Buffer (V): Deionized water (V)=1:4) .The Reconstitution buffer can be store at 4℃ for a month.

   Solution 4: Wash Buffer

   Dilute 20×Concentrated Wash Buffer with deionized water. (20×Concentrated Wash Buffer (V): Deionized water (V) = 1:19)

3. **Sample pretreatment procedure**

3.1 Pretreatment of animal tissue (chick, pig, fish, shrimp):

(1) Weigh 2.0±0.05 g of homogenate tissue sample into 50 mL centrifuge tube

(2) Add 8 mL of **Sample Extract** (solution 2), oscillate for 5 min, centrifuge at 4000 r/min at room temperature for 10 min.

(3) Take 2 mL of clear upper organic phase to a 10 mL clean and dry glass tube, water-bath to blow-dry at 50-60℃ with nitrogen evaporators/water bath

(4) Add 1 mL of **n-hexane**, oscillate for 2 min, then add 1 mL of **Reconstitution buffer** and oscillate for 30 sec. Centrifuge at 4000 r/min at room temperature for 5 min.

(5) Discard the upper n-hexane, take 50 μL of lower liquid to analysis.

Note: Sample dilution factor: 2, minimum detection dose: 0.3ppb

3.2 Pretreatment of honey:

(1) Weigh 1±0.05 g of honey into 50 mL Polystyrene centrifuge tube, add 6 mL of sample extract, oscillate for 5 min to dissolve it fully.

(2) Add 3 mL of 1×Reconstitution solution, add 11 mL of methylene dichloride, and oscillate for 5 min. Centrifuge at 4000 r/min at room temperature for 5 min.

(3) Discard the upper layer and take 8 mL of lower organic phase to another dry container, water-bath to blow-dry at 50-60℃ with nitrogen evaporators/water bath

(4) Dissolve the dry residue with 1 mL of Reconstitution buffer, add 1 mL of n-hexane, and mix for 30 sec. Centrifuge at 3000 r/min at room temperature for 5 min.

(5) Discard the upper liquid, take 50 μL of lower liquid to analysis.

Note: Sample dilution factor: 2, minimum detection dose: 0.4 ppb

3.3 Pretreatment of milk:

(1) Take 25 μL of sample solution and mix with 475 μL of 1×Reconstitution solution, oscillate for 1 min to dissolve it fully.

(2) Take 50 μL of liquid to analysis.

Note: Sample dilution factor: 20, minimum detection dose: 3 ppb

3.4 Pretreatment of milk powder:

(1) Weigh 0.5±0.02 g of homogenate sample into 10 mL Polystyrene centrifuge tube, add 5mL of deionized water, oscillate to dissolve it fully.

(2) Take 100 μL of sample and mix with 400 μL of Reconstitution buffer, oscillate for 1 min.

(3) Take 50 μL of liquid to analyze.

Note: Sample dilution factor: 50, minimum detection dose: 6 ppb

3.5 Pretreatment of egg:

(1) Weigh 1±0.02 g of homogenate sample into 10 mL Polystyrene centrifuge tube, add 5 mL of deionized water, oscillate to dissolve it fully.

(2) Take 100 μL of sample and mix with 400μL of Reconstitution buffer, oscillate for 1 min.

(3) Take 50 μL of liquid to analysis.

Note: Sample dilution factor: 30, minimum detection dose: 3 ppb

3.6 Pretreatment of urine:

(1) Take 4 mL of 1×Reconstitution solution to mix with 1mL of clear centrifugal urine sample, oscillate for 30 sec.

(2) Take 50 μL of liquid to analysis.

Note: Sample dilution factor: 5, minimum detection dose: 0.5 ppb

Assay procedure

Centrifuge the sample again after thawing before the assay. Bring all reagents to room temperature before use. All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming.

1. **Number:** number the sample and standard in order (multiple well), and keep a record of standard wells and sample wells. **Standard and Samples need test in duplicate.**
2. **Add sample:** add 50 μL of **Standard or Sample** per well, then add 50 μL of **HRP Conjugate** to each well, then add 50 μL of **antibody working solution**, cover the plate with sealer we provided, oscillate for 5 sec gently to mix thoroughly, incubate for 45 min at 25℃.
3. **Wash:** uncover the sealer carefully, remove the liquid in each well. Immediately add 300 μL of **wash buffer** to each well and wash. Repeat wash procedure for 5 times, 30 sec 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. **Color Development:** add 50 μL of **substrate solution A** to each well, and then add 50 μL of **substrate solution B**. Gently oscillate for 5 sec to mix thoroughly. Incubate shading light for 15 min at 25℃.(If the color is not clear, prolong the reaction time properly)
5. **Stop Reaction:** add 50 μL of **stop solution** to each well, oscillate gently to mix thoroughly.
6. **OD Measurement:** determine the optical density (OD value) of each well at 450 nm with a microplate reader (the 450/630 nm double wavelength is recommended).This step should be finished in 10 min after stop reaction.

Result analysis

1. **Absorbance (%)=$A/A_0 \times 100\%$**

   A: Average absorbance of standard or sample

   A_0: Average absorbance of 0 ppb Standard
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 average absorbance value of sample 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 is not brought to room temperature before use or room temperature is below 25℃.
2. During the washing procedure, if the wells turn dry, it will lead to bad linear standard curve and 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. ELISA Microtiter plate should be covered by plate sealer. Avoid the reagents to strong light.
5. **Do not use expired kit, reagents of different batches and reagents that do not belong to this kit**.
6. TMB should be abandoned if it turns color. When OD value of standard(concentration: 0)＜0.5 unit(A_{450nm}＜0.5), it indicates reagent is deteriorated.
7. Stop solution is caustic, avoid contact with skin and eyes.
8. As the OD values of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique, washing technique or temperature effects), the operator should establish a standard curve for each test.
9. Even the same operator might get different results in two separate experiments. In order to get reproducible results, the operation of every step in the assay should be controlled.
10. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
11. The kit is used for rapid screening of actual samples. If the test result is positive, the instrument method such as HPLC, LC/MS, etc. can be used for quantitative confirmation.

Storage and valid period

Store at 2~8℃ for 1 year. Avoid freeze.

Please store the opened kit at 2~8℃, protect from light and moisture. The valid period is 2 months.

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

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