8th Edition, revised in February, 2018

# SAs (Sulfonamides of 17-in-1) ELISA Kit

Catalog No: E-FS-E049

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.

## Test principle

This kit uses Indirect-Competitive-ELISA as the method. It can detect SAs (Sulfonamides of 17-in-1) in samples, such as tissue, serum, honey, milk, urine, etc. This kit is composed of ELISA Microtiter plate with pre-coated coupled antigen, HRP conjugate, antibody, standard and other supplementary reagents. During the reaction, SAs in the samples or standard competes with pre-coated coupled antigen on the ELISA Microtiter plate for anti-SAs 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 SAs. The concentration of SAs in the samples can be calculated by comparing the OD of the samples to the standard curve.

## Technical indicator

**Sensitivity:** 0.5 ppb (ng/mL)

**Reaction mode:** 25℃, 45 min~ 15 min

**Detection limit:** Tissue (high detection limit) ---0.5 ppb,
Tissue (low detection limit) ---2.5 ppb,
Swine serum/Swine urine---2 ppb,
Honey---0.5 ppb,
Milk---10 ppb

**Cross-reactivity:**

| Names | Cross-reactivity | Sensitivity(ppb) |
|---|---|---|
| Sulfamethazine (SM2) | 100% | 0.5 |
| Sulfamonomethoxine (SMM) | 670% | 0.07 |
| Sulfametoxydiazine (SMD) | 582% | 0.09 |
| Sulfadoxine (SDM') | 451% | 0.1 |
| Sulfamerazine(SM1) | 313% | 0.15 |
| Sulfadiazine (SD/SDZ) | 308% | 0.15 |
| Sulfadimetine (SM2') | 241% | 0.2 |
| Sulfadimethoxine (SDM) | 175% | 0.3 |
| Sulfamethythiadiazole (SMT) | 165% | 0.3 |
| Sulfaclozine (Esb3) | 67% | 0.8 |
| Sulfathiazole (ST) | 58% | 0.9 |
| Sulfachloropyridazine (SCPA) | 58% | 0.9 |
| Sulfamethoxypyridazine (SMP) | 57% | 0.9 |
| Sulfadimethoxine (SDT) | 60% | 0.8 |
| Sulfaquinoxaline (SQX) | 42% | 1 |
| Sulfisoxazole (SIZ) | 18% | 3 |
| Sulfamethoxazole (SMZ) | 18% | 3 |

**Sample recovery rate:** Tissue/honey---95%±25%, Swine serum/wine urine/milk ---85%±25%

## Kits components

| Item | Specifications |
|---|---|
| ELISA Microtiter plate | 96 wells |
| Standard Liquid | 1 mL each<br>(0 ppb, 0.5 ppb, 1.5 ppb, 4.5 ppb, 13.5 ppb, 40.5 ppb) |
| 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 |
| 2×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 0.01g).

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

**Reagents:** Ethyl acetate, N-hexane, acetonitrile, $Na_2HPO_4 \cdot 12H_2O$, NaOH, concentrated HCl, $NaH_2PO_4 \cdot 2H_2O$.

## Experimental preparation

Bring all reagents and samples to room temperature before use.

Open the microplate 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: 0.2 M NaOH Solution

Dissolve 0.8 g of NaOH to 100 mL of deionized water

Solution 2: 0.02 M PB Buffer

Dissolve 2.58 g of $Na_2HPO_4 \cdot 12H_2O$ and 0.44 g of $NaH_2PO_4 \cdot 2H_2O$ to 500 mL of deionized water

Solution 3: 0.5 M HCl Solution

Dissolve 4.3ml concentrated HCl,to 100 mL with deionized water.

Solution 4: Reconstitution Buffer

Dilute the 2×Reconstitution Buffer with deionized water. (2×Reconstitution Buffer (V): Deionized water (V)=1:1) .The Reconstitution buffer can be store at 4℃ for a month.

Solution 5: 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 tissue (high detection limit):**

(1) Add 3±0.05 g of homogeneous tissue sample to a centrifuge tube, then add 3 mL of 0.02 M PB buffer solution, oscillate and mix thoroughly. Add 4 ml of ethyl acetate and 2 mL of acetonitrile, oscillate for 10 min, centrifuge at above 4000 r/min for 10 min.

(2) Take 2 mL of upper liquid (about 1 g of sample), blow-dry at 50-60℃ with nitrogen evaporators/water bath.

(3) Dissolve the residual with 1 mL of N-hexane, add 1 mL of reconstitution buffer and oscillate for 1 min. Centrifuge at 4000 r/min for 5 min.

(4) Discard the upper N-hexane, take 50 μL of lower liquid for analysis.

**Note: Sample dilution factor: 1, minimum detection dose: 0.5 ppb**

**3.2 Pretreatment of tissue (low detection limit):**

(1) Add 2.0±0.05 g of homogeneous tissue sample to a centrifuge tube, then add 8 mL of 0.02 M PB buffer solution, oscillate for 2 min, centrifuge at above 4000 r/min for 10 min.

(2) Take 50 μL of liquid for analysis.

**Note: Sample dilution factor: 5, minimum detection dose: 2.5 ppb**

**3.3 Pretreatment of swine serum:**

(1) Stand blood sample at room temperature for 30 min, centrifuge at 4000 r/min for 10 min, separate the serum.

(2) Take 1 mL of serum, add 3 mL of 0.02 M PB buffer solution, and mix for 30 sec.

(3) Take 50 μL of liquid for analysis.

**Note: Sample dilution factor: 4, minimum detection dose: 2 ppb**

**3.4 Pretreatment of honey:**

(1) Weigh 1±0.05 g of honey sample into 50 mL a centrifuge tube, add 1 mL of 0.5 M HCl solution, incubate at 37℃ for 30 min.

(2) Add 2.5 mL of 0.2 M NaOH solution (**adjust the PH value to about 5**), then add 4 mL of ethyl acetate, oscillate for 5 min, centrifuge at above 4000 r/min at room temperature for 10 min.

(3) Take 2 mL of upper liquid, blow-dry at 50-60℃ with nitrogen evaporators/water bath. Add 0.5 mL of Reconstitution buffer and mix for 30 sec.

(4) Take 50 μL of liquid for analysis.

**Note: Sample dilution factor: 1, minimum detection dose: 0.5 ppb**

**3.5 Pretreatment of swine urine:**

(1) Mix 3 mL of 0.02 M PB buffer solution and 1 mL of centrifuged clear urine sample fully.

(2) Take 50 μL of liquid to analysis.

**Note: Sample dilution factor: 4, minimum detection dose: 2 ppb**

**3.6 Pretreatment of milk:**

(1) Dilute milk sample with 0.02 M PB buffer solution with the ratio of 1:19 (for example, 20 μL milk + 380 μL of 0.02 M PB buffer), mix for 30 sec.

(2) Take 50 μL of liquid to analysis.

**Note: Sample dilution factor: 20, minimum detection dose: 10 ppb**

## Assay procedure

Restore all reagents and samples 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 50μL of **HRP Conjugate** to each well, then add 50 μL of **antibody working solution**, cover the plate with sealer, 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 Reagent A** to each well, and then add 50 μL of **Substrate Reagent B**. Gently oscillate for 5 sec to mix thoroughly. Incubate with shading light for 15 min at 25℃ (The reaction time can be extended according to the actual color change).
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 micro-plate 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. The overall OD value will be lower when reagents have not been brought to room temperature before use or room temperature is below 25℃.
2. If the wells turn dry during the washing procedure, it will lead to bad linear standard curve and poor repeatability. Operate 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 (Substrate Reagent A or Substrate Reagent B) should be abandoned if it turns blue 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.
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.

**Expiry date:** expiration date is on the packing box.