

**SAs (Sulfonamides of 15-in-1) ELISA Kit**

Catalog No: E-FS-E038

96T/96T\*3

<b>Version Number:</b>	V1.1
<b>Replace version:</b>	V1.0
<b>Revision Date:</b>	2026.04.15

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.

Toll-free: 1-888-852-8623 Tel: 1-832-243-6086 Fax: 1-832-243-6017

Email: [techsupport@elabscience.com](mailto:techsupport@elabscience.com)

Website: [www.elabscience.com](http://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 Competitive-ELISA as the method for the quantitative detection. It can detect Sulfonamides of 15-in-1 (SAs) in samples, such as muscle. This kit is composed of ELISA Microtiter plate, HRP conjugate, antibody working solution, standard and other supplementary reagents. The microtiter plate in this kit has been pre-coated with coupled antigen. During the reaction, SAs in the samples or standard competes with coupled antigen on the solid phase supporter for sites of anti-SAs antibody. Then Horseradish Peroxidase (HRP) conjugate is added to each microtiter 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 SAs. The concentration of SAs in the samples can be calculated by comparing the OD of the samples to the standard curve.

## Technical indicator

**Reaction mode**(Incubation time and temperature):  $25 \pm 2^{\circ}\text{C}$ ; 30 min, 15-20 min.

**Detection limit:** Tissue Method 1 (pork), Porcine urine---approx. 40 ppb; Tissue Method 1 (chicken, fish, shrimp), bovine serum, raw milk, reconstituted milk, finished milk (pure milk, yogurt, modified milk, etc.)---approx. 20 ppb; Tissue Method 2 (fish, shrimp)---approx. 5 ppb; Tissue Method 3 (duck, chicken)---approx. 1 ppb; Pork liver, chicken liver---approx. 10 ppb; Tissue Method 1 (beef, mutton, duck), cooked food (meat products), eggs---approx. 50 ppb; honey---approx. 3 ppb.

**Cross-reactivity:** Sulfamethoxazole(SMZ)---100%; Sulfadiazine(SD)---22%; Sulfamerazine(SM1)---49%; Sulfaquinoxaline(SQX)---63%; Sulfadimidine(SM2)---40% Sulfamonomethoxine (SMM)--- 163%; Sulfadimethoxine (SDM) ---392%; Sulfathiazole(ST)---51%; Sulfamethoxyipyridazine(SMP)---178%; Sulfapyridine(SPD)---178%; Sulfamethoxydiazine(SMD)---189%; Sulfachloropyridazine(SCP)---38%; Sulfanitran(SNT)---495%; Sulfisomidine(SIM)---90%; Sulfamethizole(SMT)---40%

**Sample recovery rate:**  $90 \pm 30\%$ .

**Kits components**

Item	Specifications
ELISA Microtiter plate	96 wells
Standard Liquid	1.5 mL each (ppb=ng/mL=ng/g) (0 ppb, 2 ppb, 6 ppb, 18 ppb, 54 ppb, 162 ppb)
HRP Conjugate	7 mL
Antibody Working Solution	10 mL
Substrate Reagent A	7 mL
Substrate Reagent B	7 mL
Stop Solution	7 mL
20×Concentrated Wash Buffer	25 mL
20×Concentrated Sample Diluent	50 mL
Plate Sealer	1 piece
Sealed Bag	1 piece
Manual	1 copy

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

**Other materials required but not supplied**

**Instruments:** Microplate reader, Printer, Homogenizer, Nitrogen evaporators, Water bath, Vortex mixer, Centrifuge, Graduated pipette, Balance (sensitivity 0.01g).

**Micropipette:** Single channel (20-200  $\mu$ L, 100-1000  $\mu$ L), Multichannel (30-300  $\mu$ L).

**Reagents:**  $K_4[Fe(CN)_6] \cdot 3H_2O$ ,  $ZnSO_4 \cdot 7H_2O$ ,  $Na_2HPO_4 \cdot 12H_2O$ ,  $NaH_2PO_4 \cdot 2H_2O$ , NaOH, Ethyl Acetate (Analytical Grade), N-Hexane (Analytical Grade), Anhydrous Methanol.

---

**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°C.
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. FOR RESEARCH USE ONLY. ELISA Microtiter plate should be covered by plate sealer. Avoid the kit to strong light.
5. **Each reagent is optimized for use in the E-FS-E038. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other E-FS-E038 with different lot numbers.**
6. Substrate Reagent should be abandoned if it turns blue color. When OD value of standard (concentration: 0) < 0.8 unit (A450nm < 0.8), it indicates the reagent may be deteriorated.
7. If crystallization occurs in the Antibody Working Solution or 20×Concentrated Sample Diluent, warm to room temperature until crystals are completely dissolved before use!!
8. Stop solution is caustic, avoid contact with skin and eyes.
9. 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.
10. 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.
11. **For mentioned sample fast and efficient extraction methods are included in the kit description. Please consult technical support for the applicability if other sample need to be tested.**
12. 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 expiry date**

Store the kit at 2-8°C. Do not freeze any test kit components.

Return any unused microwells to their original foil bag and reseal them together with the desiccant provided and further store at 2-8°C. After opening, the kit is stable for up to 1 month.

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

---

## Experimental preparation

Restore 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

*Please prepare solution according to the number of samples. Don't use up all components in the kit at once!*

#### Solution 1: $\text{K}_4[\text{Fe}(\text{CN})_6]\cdot 3\text{H}_2\text{O}$ Solution

Dissolve 1.52 g of  $\text{K}_4[\text{Fe}(\text{CN})_6]\cdot 3\text{H}_2\text{O}$  to 10 mL with deionized water.

#### Solution 2: $\text{ZnSO}_4$ Solution

Dissolve 2.88 g of  $\text{ZnSO}_4\cdot 7\text{H}_2\text{O}$  to 8.64 mL with deionized water.

#### Solution 3: **PB** Solution

Dissolve 6 g of  $\text{Na}_2\text{HPO}_4\cdot 12\text{H}_2\text{O}$  and 0.5 g of  $\text{NaH}_2\text{PO}_4\cdot 2\text{H}_2\text{O}$  to 300 mL with deionized water, mix fully.

#### Solution 4: **1 M NaOH** Solution

Dissolve 4 g of **NaOH** to 100 mL with deionized water, mix fully.

#### Solution 5: **Sample Reconstitution Buffer**

Add 5 mL **Anhydrous Methanol** to 95 mL deionized water, mix fully.

#### Solution 6: **Sample Diluent**

Dilute **20×Concentrated Sample Diluent** with deionized water. (20×Concentrated Sample Diluent (V): Deionized water (V) = 1:19).

#### Solution 7: **Wash Buffer**

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

### 3. Sample pretreatment

*Deteriorated samples may cause false negative results and low recovery rates. It is recommended to use fresh samples.*

#### 3.1 Pretreatment of porcine urine sample:

- (1) Take the urine sample to be tested. If the sample is turbid, centrifuge at 4000 r/minute for 5 minutes or filter.
- (2) Take 20  $\mu$ L of the supernatant for analysis.

**Note: Sample dilution factor: 1, detection limit: 40 ppb.**

#### 3.2 Pretreatment of raw milk, reconstituted milk, finished milk (pure milk, yogurt, modified milk, etc.) sample:

- (1) **Reconstituted milk:** Weigh  $1 \pm 0.05$  g of milk powder into a clean centrifuge tube, add 8 mL of deionized water, and vortex until the milk powder is completely dissolved.  
**Acidic samples such as yogurt:** Weigh  $1 \pm 0.01$  g of sample, and adjust the pH to neutral with approximately 50  $\mu$ L of **1 M NaOH Solution** (Solution 4).
- (2) Weigh  $1 \pm 0.05$  g of fresh sample into a 4 mL centrifuge tube, add 100  $\mu$ L of **ZnSO<sub>4</sub> Solution** (Solution 2), 100  $\mu$ L of **K<sub>4</sub>[Fe(CN)<sub>6</sub>]•3H<sub>2</sub>O Solution** (Solution 1), vortex for 1 minute.
- (3) Add 1.8 mL of **PB Solution** (Solution 3), vortex for 1 minute. Centrifuge at 4000 r/minute for 5 minutes.
- (4) Take 200  $\mu$ L of the upper clear liquid in another tube and add 200  $\mu$ L of deionized water, vortex for 1 minute.
- (5) Take 20  $\mu$ L for analysis.

**Note: Sample dilution factor: 6, detection limit: 20 ppb.**

#### 3.3 Pretreatment of tissue method 1 (pork, chicken, fish, shrimp, beef, mutton, duck) sample:

- (1) Remove fat from sample, homogenize the sample with homogenizer.
- (2) Weigh  $1 \pm 0.05$  g of homogenate fresh muscle sample into a centrifuge tube, add 9.5 mL of deionized water, add 0.5 mL of **20×Concentrated Sample Diluent**, vortex for 1 minute and mixed fully.

*Note: The organization must be completely dispersed!*

- (3) Centrifuge at 4000 r/minute for 10 minutes.
- (4) Take 20  $\mu$ L for analysis (avoid aspirating upper-layer impurities).

**Note: Sample dilution factor: 10, detection limit: pork--40 ppb; chicken, fish, shrimp--20 ppb; beef, mutton, duck--50 ppb.**

#### 3.4 Pretreatment of eggs sample:

- (1) Transfer 200  $\mu$ L of the homogenized fresh eggs sample into a centrifuge tube, add 0.8 mL of deionized water, vortex for 1 minute.
- (2) Take 20  $\mu$ L for analysis.

**Note: Sample dilution factor: 5, detection limit: 50 ppb.**

**3.5 Pretreatment of tissue method 2 (fish, shrimp) sample:**

- (1) Remove fat from sample, homogenize the sample with homogenizer.
- (2) Weigh  $2\pm 0.05$  g of homogenate muscle fresh sample into a 50 mL centrifuge tube, add 0.5 mL of deionized water, 8 mL of **Ethyl Acetate**, vortex for 1 minute.

*Note: The organization must be completely dispersed!*

- (3) Shake the tube at 300 rpm for 20 minutes. Centrifuge at 4000 r/minute for 10 minutes.
- (4) Take 4 mL of the upper clear liquid in another tube and dry at 50-60°C with nitrogen evaporators or water bath. (Please do it in a ventilated environment.)
- (5) Add 2 mL of **N-hexane**, then add 1 mL of **Sample Reconstitution Buffer** (Solution 5). Vortex for 30 s and centrifuge at 4000 r/minute for 5 minutes.
- (6) Completely discard the upper n-hexane layer and impurities in the middle layer, and stand for 20–25 minutes to allow n-hexane to evaporate completely.
- (7) Take 20  $\mu$ L for analysis.

**Note: Sample dilution factor: 1, detection limit: 5 ppb.**

**3.6 Pretreatment of tissue method 3 (duck, chicken) sample:**

- (1) Remove fat from sample, homogenize the sample with homogenizer.
- (2) Weigh  $2\pm 0.05$  g of homogenate muscle fresh sample into a 50 mL centrifuge tube, add 4 mL of **Sample Diluent** (Solution 6), vortex for 2 minutes. Add 8 mL of **Ethyl Acetate**, vortex for 2 minutes.
- (3) Centrifuge at 4000 r/minute for 10 minutes.
- (4) Take 4 mL of the upper clear liquid in another tube and dry at 50-60°C with nitrogen evaporators or water bath. (Please do it in a ventilated environment.)
- (5) Add 2 mL of **N-hexane**, then add 0.5 mL of **Sample Reconstitution Buffer** (Solution 5). Vortex for 1 minute and centrifuge at 4000 r/minute for 5 minutes.
- (6) Completely discard the upper n-hexane layer and impurities in the middle layer, and stand for 20–25 minutes to allow n-hexane to evaporate completely.
- (7) Take 20  $\mu$ L for analysis.

**Note: Sample dilution factor: 0.5, detection limit: 1 ppb.**

**3.7 Pretreatment of honey sample:**

- (1) Weigh  $2\pm 0.05$  g of homogenate muscle fresh sample into a 50 mL centrifuge tube, add 4 mL of **Sample Diluent** (Solution 6), vortex for 2 minutes until the honey is completely dissolved.
- (2) Add 8 mL of **Ethyl Acetate**, vortex for 2 minutes. Centrifuge at 4000 r/minute for 10 minutes.
- (3) Take 4 mL of the upper clear liquid in another tube and dry at 50-60°C with nitrogen evaporators or water bath. (Please do it in a ventilated environment.)
- (4) Add 0.5 mL of **Sample Reconstitution Buffer** (Solution 5). Vortex for 1 minute.
- (5) Take 20  $\mu$ L for analysis.

**Note: Sample dilution factor: 0.5, detection limit: 3 ppb.**

**3.8 Pretreatment of cooked food (meat products) sample:**

- (1) Weigh  $1\pm 0.05$  g of homogenate fresh sample into a 50 mL centrifuge tube, add 10 mL of deionized water, vortex for 1 minute.

*Note: The organization must be completely dispersed!*

- (2) Centrifuge at 4000 r/minute for 10 minutes.
- (3) Take 20  $\mu$ L of the supernatant for analysis (Avoid aspirating lower-layer impurities.).

**Note: Sample dilution factor: 10, detection limit: 50 ppb.**

**3.9 Pretreatment of bovine serum sample:**

- (1) Collect animal blood and place it in a glass tube to allow coagulation. Centrifuge at 3000 g for 5 min, then collect the upper clear liquid (serum). If insufficient serum volume results from no obvious contraction of the blood clot, gently dislodge the clot with a wooden stick before centrifugation.

*Note: Hemolysis should be avoided as much as possible during blood collection. If the serum is turbid, the supernatant may be obtained by centrifugation for testing!*

- (2) Take 20  $\mu$ L of the supernatant for analysis.

**Note: Sample dilution factor: 1, detection limit: 20 ppb.**

**3.10 Pretreatment of pork liver, chicken liver sample:**

- (1) Remove fat from sample, homogenize the sample with homogenizer.
- (2) Weigh  $1\pm 0.05$  g of fresh sample into a 50 mL centrifuge tube, add 2 mL of **PB Solution** (Solution 3), vortex for 1 minute.
- (3) Centrifuge at 4000 r/minute for 10 minutes.
- (4) Take 20  $\mu$ L of the supernatant for analysis (Avoid aspirating lower-layer impurities.).

**Note: Sample dilution factor: 3, detection limit: 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. The unused ELISA Microtiter plate should be sealed as soon as possible and stored at 2-8°C.

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 20 µL of **Standard or Sample** per well, then add 50 µL of **HRP Conjugate** to each well, then add 80 µL of **Antibody Working Solution**, cover the plate with plate sealer. Oscillate for 10 s gently to mix thoroughly, incubate at 25±2°C for 30 min in shading light.
3. **Wash:** uncover the sealer carefully, remove the liquid in each well. Immediately add 260 µL of **Wash Buffer** (Solution 7) to each well, repeat wash procedure for 4 times, 15-30 s 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 100 µL of Substrate mixed solution to each well (**Substrate Reagent A** and **Substrate Reagent B** are fully mixed at ratio 1:1 by volume, the mixture should be used within 5 min, avoid using metal containers or stirring the reagents), Gently oscillate for 10 s to mix. thoroughly. Incubate at 25±2°C for 15-20 min in shading light.
5. **Stop Reaction:** add 50 µL of **Stop Solution** to each well, Gently oscillate for 10 s to mix thoroughly.
6. **OD Measurement:** determine the optical density (OD value) of each well at 450 nm (reference wavelength 630 nm) with a microplate reader. This step should be finished in 5 min after stop reaction.

## Result analysis

### 1. Absorbance% = $A/A_0 \times 100\%$

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 form for accurate and fast analysis on a large number of samples.

### Sulfonamides of 15-in-1 (E-FS-E038) Standard Curve

