SAs (Sulfonamides) ELISA Kit

Catalog No: E-FS-E162

96T/96T*3

Version Number:	V1.4
Replace version:	V1.3
Revision Date:	2025.09.08

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
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 Competitive-ELISA as the method for the quantitative detection. It can detect Sulfonamides in samples, such as muscle, honey, egg etc. 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, SA_S in the samples or standard competes with coupled antigen on the solid phase supporter for sites of anti-SA_S antibody. Then Horseradish Peroxidase (HRP) conjugate is added to each microtiter plate well, and substrate reagent is added for color development. There is a negative correlation between the OD value of samples and the concentration of SA_S. The concentration of SA_S 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℃; 30 min, 15 min

Detection limit: Urine (pig), Muscle (method 1) (pig) ---40 ppb; Muscle (method 1)(chicken, fish, shrimp), serum, raw milk, milk power, yogurt ---20 ppb; Muscle (method 2) (Pork, fish, shrimp, chicken, duck, beef, mutton)---2 ppb; Egg---5 ppb; Liver (pig, chicken)---10 ppb; Muscle (method 1)(duck, beef, mutton), cooked food (meat products), feed ---50 ppb; Honey---3 ppb.

Cross-reactivity:

Names	Cross-reactivity
Sulfamethazine (SM_2)	40%
Sulfamonomethoxine (SMM)	163%
Sulfametoxydiazine (SMD)	189%
Sulfamerazine(SM_1)	49%
Sulfadimethoxine (SDM)	392%
Sulfamethythiadiazole (SMT)	40%
Sulfathiazole (ST)	51%
Sulfachloropyridazine (SCP)	38%
Sulfamethoxypyridazine (SMP)	178%
Sulfaquinoxaline (SQX)	63%
Sulfapyridine (SPD)	178%
Sulfanitran (SNT)	495%
Sulfisomidine (SIM)	90%

Sample recovery rate: 90%±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	3 pieces
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 (sensibility 0.01g).

Micropipette: Single channel (20-200 µL, 100-1000 µL), Multichannel (30-300 µL).

Reagents: N-hexane, Acetonitrile, $Na_2HPO_4 \cdot 12H_2O$, NaOH, Phosphoric Acid, $NaH_2PO_4 \cdot 2H_2O$, Methanol, $ZnSO_4 \cdot 7H_2O$, $K_4[Fe(CN)_6] \cdot 3H_2O$, Trichloroacetic Acid

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. FOR RESEARCH USE ONLY. ELISA Microtiter plate should be covered by plate sealer. Avoid th e kit to strong light.
5. **Each reagent is optimized for use in the E-FS-E162. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other E-FS-E162 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 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. **For mentioned sample fast and efficient extraction methods are included in the kit descriptio- n. Please consult technical support for the applicability if other sample need to be tested.**
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.
12. If the samples used are deteriorated, false negatives may occur during testing, and the recovery rate will be low; it is recommended to use fresh samples.
13. When adding sample, HRP conjugate, antibody working solution, substrate A&B mixture, and stop solution to the wells, each step must not exceed 3 min.
14. Metal-containing materials should be avoided for storing and stirring reagents.

Storage and expiry date

Store the kit at 2-8℃. 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℃.

Expiry date: expiration date is on the packing box.

Experimental preparation

Restore all reagents and samples to room temperature (25℃) 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: $K_4[Fe(CN)_6]$ Solution

Dissolve 1.52 g of **$K_4[Fe(CN)_6]\cdot 3H_2O$** with 10 mL deionized water.

Solution 2: $ZnSO_4$ Solution

Dissolve 2.88 g of **$ZnSO_4\cdot 7H_2O$** with 8.64 mL deionized water.

Solution 3: PB Solution

Dissolve 6 g of **$Na_2HPO_4\cdot 12H_2O$** and 0.5 g of **$NaH_2PO_4\cdot 2H_2O$** with 300 mL deionized water.

Solution 4: Liver extract solution

Dissolve 1 g of **Trichloroacetic Acid** with 100 mL deionized water.

Solution 5: 1 M NaOH Solution

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

Solution 6: Phosphoric acid solution

Dissolve 2 mL **Phosphoric Acid (Analytical pure, ≥85%)** with 98 mL deionized water.

Solution 7: Wash Buffer

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

Solution 8: Sample reconstitution solution

Dilute **20×Concentrated Sample Diluent** with deionized water. (20×Concentrated Wash Buffer (V): Deionized water (V) = 1:3).

3. Sample pretreatment procedure

3.1 Pretreatment of urine (pig) sample:

(1) Take the urine sample for testing. If the sample is cloudy, centrifuge at 3000 g or more for 5 min or filter it.

(2) Take 20 µL of the supernatant for testing.

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

3.2 Pretreatment of milk (milk power, yogurt, raw milk etc.)sample:

(1) **Milk power**: Weigh 1 ± 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.

For yogurt and other acidic samples: Weigh 1 ± 0.01 g of the sample and adjust the pH to neutrality using 1 M NaOH solution (solution 5) (approximately 50 µL).

(2) Take 1 mL (or 1 g) of the sample to be tested and place it in a 4 mL centrifuge tube.

(3) Add 100 µL of $ZnSO_4$ Solution (solution 2) and 100 µL of $K_4[Fe(CN)_6]$ solution (solution 1), then vortex thoroughly for 30 s.

(4) Add 1.8 mL of PB solution (Solution 3), then vortex thoroughly for 1 min.
(5) Centrifuge at 4000 g or more for 5 min.
(6) Take 200 µL of the supernatant and add 200 µL of deionized water, then mix thoroughly.
(7) Take 20 µL for testing.

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

3.3 Pretreatment of muscle (method 1) (Pig, chicken, fish, shrimp, beef, mutton, duck) sample:

(1) Weigh 1.00 ± 0.05 g of homogenized fresh tissue sample into a 50 mL centrifuge tube.
(2) Add 9.5 mL of deionized water and 0.5 mL of 20×concentrated sample diluent successively, then vortex thoroughly for 1 min. Ensure the tissue is completely dispersed.
(3) Centrifuge at 4000 g or more for 10 min.
(4) Immediately take 20 µL of the supernatant for testing. Avoid drawing in any upper impurities.

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

3.4 Pretreatment of muscle (method 2) (Pig, fish, shrimp, chicken, duck, beef, mutton), egg sample:

(1) Weigh 2 ± 0.05 g of the homogenized sample into a 50 mL centrifuge tube.
(2) Add 0.1 mL of phosphoric acid solution (Solution 6) and 6 mL of acetonitrile sequentially, then immediately vortex thoroughly for 2 min.
(3) Centrifuge at 4000 g or higher for 5 min.
(4) Transfer 2 mL of the supernatant into a new 4 mL centrifuge tube.
(5) Place the centrifuge tube in a water bath at 60-70℃ and blow-dry with nitrogen.
(6) Add 1 mL of hexane, vortex thoroughly for 30 s, then add 0.5 mL of sample reconstitution solution (Solution 8), and vortex gently for 30 s.
(7) Centrifuge at 4000 g or higher for 5 min.
(8) Completely discard the upper hexane layer and the intermediate layer of impurities.
(9) Take 20 µL for analysis.

Note: Sample dilution factor: 1, detection limit: Pig, fish, shrimp, chicken, duck, beef, mutton ---2 ppb; egg ---5 ppb

3.5 Liver (pig, chicken)

(1) Weigh 2 ± 0.05 g of the homogenized sample into a 50 mL centrifuge tube.
(2) Add 3 mL of the Wash Buffer (Solution 7), followed by 3 mL of Liver extract solution (Solution 4). Vortex vigorously for 1 min.
(3) Centrifuge at 4000 g for 5 min.
(4) Take 1 mL of the middle clear layer to a new centrifuge tube.

Note: Avoid aspirating the upper or lower solid layers, as this will affect the test results.

(5) Add 20 µL of 1 M NaOH solution (Solution 5) and vortex for 10 s to mix well.
(6) Centrifuge at 4000 g for 5 min.

(7) Take 20 µL of the supernatant for analysis.

Note: Sample dilution factor: 4, detection limit: 10ppb

3.6 Pretreatment of cooked food (meat products), feed sample:

(1) Weigh 1 ± 0.05 g of the homogenized sample into a 50 mL centrifuge tube.
(2) Add 10 mL of deionized water and vortex thoroughly for 1 min until completely dispersed.
(3) Centrifuge at 4000 g for 10 min.
(4) Carefully take 20 µL of the supernatant for detection.

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

3.7 Pretreatment of serum sample:

(1) Add 1 mL of the sample into a centrifuge tube.
(2) Sequentially add 1 mL of methanol and 1 mL of wash buffer (Solution 7). Vortex thoroughly for 30 s.
(3) Centrifuge at 4000 g for 5 min.
(4) Take 20 µL of the supernatant for analysis.

Note: Sample dilution factor: 3, detection limit: 20 ppb

3.8 Pretreatment of honey sample:

(1) Weigh 2 ± 0.05 g of the homogenized sample into a 50 mL centrifuge tube.
(2) Add 1 mL of deionized water and vortex until the honey is completely dissolved.
(3) Sequentially add 0.1 mL of phosphoric acid solution (Solution 6) and 5 mL of acetonitrile. Immediately vortex vigorously for 2 min.
(4) Centrifuge at 4000 g for 5 min.
(5) Transfer 2 mL of the supernatant to a new 4 mL centrifuge tube.
(6) Evaporate to dryness under a gentle stream of nitrogen in a 60-70 ℃ water bath.
(7) Add 1 mL of n-hexane and vortex vigorously for 30 s. Then add 0.5 mL of sample reconstitution solution (Solution 8) and vortex gently at low speed for 30 s.
(8) Centrifuge at 4000 g for 5 min.
(9) Completely discard the upper hexane layer and interfacial impurities.
(10) Take 20 µL (of the lower aqueous phase) for detection.

Note: Sample dilution factor: 1, detection limit: 3 ppb

Assay procedure

Restore all reagents and samples to room temperature (25℃)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℃.

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** to each well, cover the plate with plate sealer, oscillate for 5s gently to mix thoroughly, incubate at 25℃ with for 30 min shading light.

3. **Wash:** uncover the sealer carefully, remove the liquid in each well. Immediately add 260 μL of **Wash Buffer** to each well and wash. Repeat wash procedure for 4 times, 30 s intervals/time. Invert the plate and pat it against thick clean with absorbent paper (If bubbles exist in the wells, clean tips can be used to prick them).
4. **Color Development:** add 100 μL mixture of **Substrate Reagent A** and **Substrate Reagent B** to each well. Gently oscillate for 5 s to mix thoroughly. Incubate at 25℃ for 15 min with shading light (The reaction time can be extended according to the actual color change).

   Note: Mix Substrate Reagent A and Substrate Reagent B at a 1:1 volume ratio, it must be thoroughly mixed. The mixture should be used within 5 min.
5. **Stop Reaction:** add 50 μL of **Stop Solution** to each well. Gently oscillate 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 10 min after stop reaction.

Result analysis

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

   A: Average absorbance of standard or sample

   A0: 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 form for accurate and fast analysis on a large number of samples.

Sulfonamides (E-FS-E162) **Standard Curve**