SAs (Sulfonamides) ELISA Kit

Catalog No: E-FS-E162

96T/96T*3

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.

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Test principle

This kit uses Competitive-ELISA as the method for the quantitative detection. It can detect total sulfonamides (SAs) in samples, such as muscle, feed, etc. This kit is composed of ELISA Microtiter plate, HRP conjugate, antibody working solution, standard and other supplementary reagents. The microtiter plate provided in this kit has been pre-coated with coupled antigen. During the detection, 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 well, and substrate reagent 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℃; 30 min, 15 min.

Detection limit: Urine, Muscle (methond 1, pork) ---40 ppb; Liver---10 ppb; Honey ---3 ppb; Muscle (methond 1, chicken, fish, shrimp), Serum, Raw milk, Finished Milk---20 ppb; Muscle (methond 2, eggs) ---2 ppb; Muscle (methond 1, beef, mutton, duck), Feed ---50 ppb.

Cross-reactivity:

Names	Cross-reactivity
Sulfamethoxazole(SMZ)	100%
Sulfadiazine (SD)	22%
Sulfamethazine (SM_2)	40%
Sulfamerazine(SM1)	49%
Sulfaquinoxaline(SQX)	63%
Sulfamonomethoxine (SMM)	>100%
Sulfadimethoxine (SDM)	>100%
Sulfathiazole(ST)	51%
Sulfamethoxypyridazine(SMP)	>100%
Sulfapyridine(SPD)	>100%
Sulfametoxydiazine (SMD)	>100%
Sulfachloropyridazine(SCP)	38%
sulfanitran (SNT)	>100%
Sulfisomidine(SIM)	90%
Sulfamethythiadiazole (SMT)	40%

Sample recovery rate: 90%±30%

Kits components

Item	Specifications
ELISA Microtiter plate	96 wells
Standard Liquid	1 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	6 mL
Substrate Reagent B	6 mL
Stop Solution	6 mL
20×Concentrated Wash Buffer	25 mL
20×Concentrated Sample Solution	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, Homogenizer, Vortex mixer, Centrifuge, Nitrogen evaporators, Water bath, Balance (sensibility 0.01 g).

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

Reagents: $K_4Fe(CN)_6 \cdot 3H_2O$, $ZnSO_4 \cdot 7H_2O$, $Na_2HPO_4 \cdot 12H_2O$, $NaH_2PO_4 \cdot 2H_2O$, Trichloroacetic Acid ($C_2HCl_3O_2$), NaOH, Concentrated H_3PO_4, Acetonitrile, N-hexane, 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℃.
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 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. **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.

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 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: $K_4Fe(CN)_6$ Solution *(for raw milk, finished milk sample)*

   Dissolve 1.52 g of **$K_4Fe(CN)_6 \cdot 3H_2O$** with 10 mL of deionized water, mix fully.

   Solution 2: $ZnSO_4$ Solution *(for raw milk, finished milk sample)*

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

   Solution 3: PB Solution *(for raw milk, finished milk sample)*

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

   Solution 4: Liver Extracting Solution *(for chicken, swine sample)*

   Dissolve 1 g of **$C_2HCl_3O_2$** with 100 mL of deionized water, mix fully.

   Solution 5: 1 M NaOH Solution

   (for raw milk, finished milk, chicken, swine sample)

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

   Solution 6: H_3PO_4 Solution *(for fish, shrimp, livestock, honey sample)*

   Add 2 mL of **Concentrated H_3PO_4** to 98 mL of deionized water, mix fully.

   Solution 7: Sample Solution *(for fish, shrimp, livestock, honey sample)*

   Dilute the **20×Concentrated Sample Solution** with deionized water (20×Concentrated Sample Solution (V): Deionized water (V) =1:3).

   Solution 8: 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 urine (swine) sample:

(1) Take urine sample, (if the urine sample is turbid, it should be filtered or centrifuged at 4000 r/min for 5 min until the urine sample become clear).

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

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

3.2 Pretreatment of raw milk, finished milk sample:

(1) **Reconstituted milk** (powder): Weigh 1±0.05 g of sample into a centrifuge tube. Add 8 mL of deionized water. Immediately vortex for 30 s, mix fully.

Acidic samples such as yogurt: Weigh 1±0.05 g of homogenate sample into a centrifuge tube. Add **1 M NaOH Solution** (Solution 5) (about 50 µL) for adjust PH=7.

(2) Take 1 mL (1 g) of sample into a 4 mL centrifuge tube, add 100 µL of **$ZnSO_4$ Solution ((**Solution 2) and add 100 µL of **$K_4Fe(CN)_6$ Solution** (Solution 1). Immediately vortex for 30 s, mix fully.

(3) Add 1.8 mL of **PB Solution** (Solution 3). Centrifuge at 4000 rpm for 5 min at room temperature.

(4) Take 200 µL of the supernatant to 200 µL of deionized water, mix fully.

(5) Take 20 µL for analysis.

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

3.3 Pretreatment muscle (methond 1) sample:

(1) Remove fat from sample, homogenize the sample with homogenizer.

(2) Weigh 1.00±0.05 g of homogenate fresh sample into a 50 mL centrifuge tube. Add 9.5 mL of deionized water and add 0.5 mL of **20×Concentrated Sample Solution**. Immediately vortex for 1 min, mix fully.

(3) Centrifuge at 4000 rpm for 10 min at room temperature.

(4) Take 20 µL for the supernatant analysis.

Note: Sample dilution factor: 10, detection limit: Pork ---40 ppb;
Fish, Shrimp, Chicken---20 ppb;
Beef, Mutton, Duck---50 ppb.

3.4 Pretreatment of muscle (methond 2) sample:

(1) Remove fat from sample, homogenize the sample with homogenizer.

(2) Weigh 2±0.05 g of homogenate sample into a 50 mL centrifuge tube. Add 0.1 mL of **H_3PO_4 Solution** (Solution 6) and add 6 mL of **Acetonitrile**. Immediately vortex for 2 min, mix fully.

(3) Centrifuge at 4000 rpm for 5 min at room temperature.

(4) Remove 2 mL of the supernatant to a 4 mL centrifuge tube, dry at 60-70℃ with nitrogen evaporators or water bath.(Please do it in a ventilated environment.)

(5) Dissolve the residue with 1 mL of **N-hexane**, immediately vortex for 30 s and 0.5 mL of **Sample Solution** (Solution 7). Vortex for 30 s

(6) Centrifuge at 4000 rpm for 5 min at room temperature. Remove the upper layer of N-hexane and intermediate layer impurities.

(7) Take 20 µL for analysis.

Note: Sample dilution factor: 1, detection limit:Egg--- 2 ppb

3.5 Pretreatment of liver (chicken, swine) sample:

(1) Remove fat from sample, homogenize the sample with homogenizer.

(2) Weigh 2.00±0.05 g of homogenate sample into a 50 mL centrifuge tube. Add 3 mL of **Wash Buffer** (Solution 8) and add 3 mL of **Liver Extracting Solution** (Solution 4). Immediately vortex for 1 min, mix fully.

(3) Centrifuge at 4000 rpm for 5 min at room temperature.

(4) Take 1 mL of the intermediate layer solution to a new centrifuge tube. Add 20 µL of **1 M NaOH Solution** (Solution 5), immediately vortex for 10 s. Centrifuge at 4000 rpm for 5 min at room temperature.

(5) Take 20 µL of supernatant for analysis.

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

3.6 Pretreatment of feed sample:

(1) Homogenize the representative sample with a homogenizer and mix fully.

(2) Weigh 1±0.05 g of homogenate sample into a 50 mL centrifuge tube. Add 10 mL of deionized water. Immediately vortex for 1 min, mix fully.

(3) Centrifuge at 4000 rpm for 10 min at room temperature.

(4) Take 20 µL of supernatant for analysis.

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

3.7 Pretreatment of serum sample:

(5) Weigh 1±0.05 g (1mL) of homogenate sample into a centrifuge tube. Add 1 mL of **Methanol** and 1 mL of **Wash Buffer** (Solution 8). Immediately vortex for 1 min, mix fully.

(6) Centrifuge at 4000 rpm for 5 min at room temperature.

(7) Take 20 µL of supernatant for analysis.

Note: Sample dilution factor: 3, detection limit: Serum---20 ppb;

3.8 Pretreatment of honey sample:

(1) Weigh 2±0.05 g of homogenate sample into a 50 mL centrifuge tube. Dissolve honey with 1 mL of deionized water, vortex for 5 min.

(2) Add 0.1 mL of **H_3PO_4 Solution** (Solution 6) and add 5 mL of **Acetonitrile**. Immediately vortex for 2 min, mix fully. Centrifuge at 4000 rpm for 5 min at room temperature.

(3) Remove 2 mL of the supernatant to 4 mL centrifuge tube, dry at 60-70℃ with nitrogen evaporators or water bath.

(4) Add 1 mL of **N-hexane**, immediately vortex for 30 s and 0.5 mL of **Sample Solution** (Solution 7). Vortex for 30 s.

(5) Centrifuge at 4000 rpm for 5 min at room temperature. Remove the upper layer of N-hexane and intermediate layer impurities.

(6) Take 20 µL for analysis.

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, add 50 μL of **HRP Conjugate**, then Add 80 μL of **Antibody Working Solution** into each well. Gently oscillate for 10 s to mix thoroughly and cover the plate with sealer. Incubate at 25℃ 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 8) to each well and wash. Repeat the wash procedure for 4 times, 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 50 μL of **Substrate Reagent A** to each well, and then add 50 μL of **Substrate Reagent B**. Gently oscillate for 10 s to mix thoroughly. Incubate at 25℃ for 15 min in shading light (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 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_0×100%**

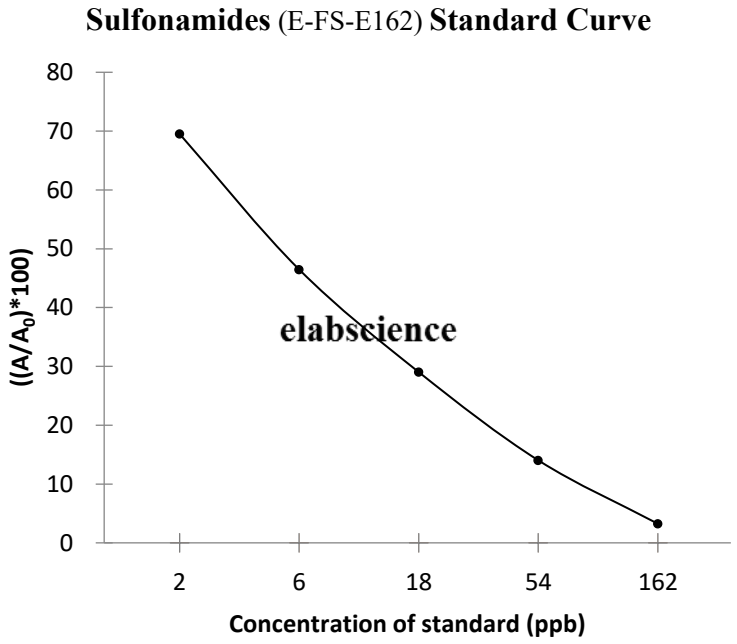
   A: Average absorbance of standard or sample

   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 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 of batch samples.



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