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Datasheet

Ver.1 Date : 20190328

SAs (Total Sulfonamides) ELISA Kit

Cat# E5022

Storage at 2-8˚C for 1 year

SPECIFICATION

Sensitivity: 2 ppb (ng/mL)

Reaction mode: 25℃, 30 min~15 min

Detection limit:

Urine, Tissue (methond 1, Pork) ---40 ppb; Liver---10 ppb; Honey ---3 ppb;

Tissue (methond 1, Chicken, fish, shrimp), Serum, Raw Milk, Reconstituted Milk,

Finished Milk---20 ppb; Tissue (methond 2) ---2 ppb;

Tissue (methond 1, beef, mutton, duck), Eggs, Feed ---50 ppb.

Cross-reactivity:

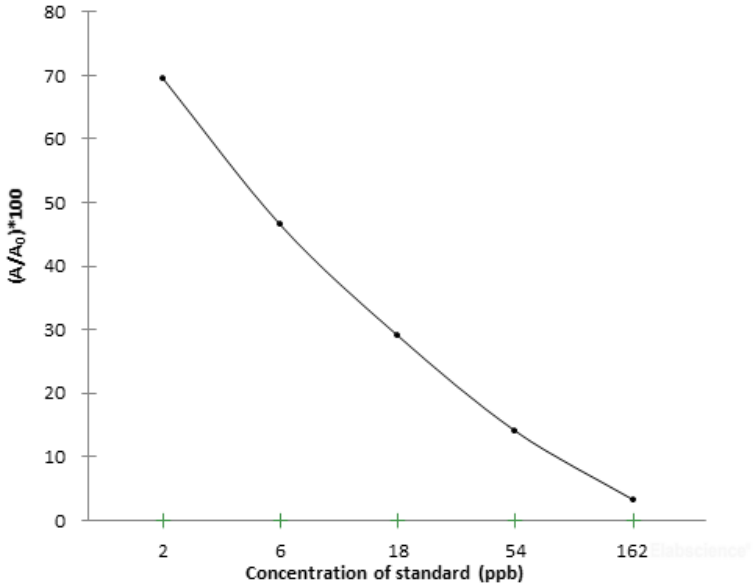
Names	Cross-reactivity
Sulfamethazine (SM2)	40%
Sulfamonomethoxine (SMM)	163%
Sulfametoxydiazine (SMD)	189%
Sulfadimethoxine (SDM)	392%
Sulfamerazine(SM1)	49%
Sulfadiazine (SD)	22%
sulfanitran (SNT)	495%
Sulfamethythiadiazole (SMT)	40%
Sulfathiazole(ST)	51%
Sulfachloropyridazine(SCP)	38%
Sulfamethoxypyridazine(SMP)	178%
Sulfaquinoxaline(SQX)	63%
Sulfisomidine(SIM)	90%
Sulfamethoxazole(SMZ)	100%

Sample recovery rate: 90%±30%.

PRINCIPLE of KIT

This kit uses Competitive-ELISA as the method. It can detect total sulfonamides (SAs) in samples, such as tissue, 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.

KITS COMPONENTS



Item	Specifications
ELISA Microtiter Plate	96 wells
Standard Liquid	1 mL each (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

Instrument: Microplate Reader, Homogenizer, Oscillators, Centrifuge, Nitrogen Evaporators, Water bath, Balance (sensibility 0.01 g)

Micropipettor: Single-channel (20-200 µL, 100-1000 µL), Multi-channel (300 µL).

Reagents: K4 [Fe (CN)6]·3H2O, ZnSO4 ·7H2O, Na2HPO4·12H2O, NaH2PO4·2H2O, Trichloroacetic Acid (C2HCl3O2), NaOH, Concentrated H3PO4 (AR, ≥85%), Acetonitrile, N-hexane, Methanol.

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

   Solution 1: K4 [Fe (CN)6] Solution Dissolve 1.52 g of K4 [Fe (CN)6]·3H2O with 10 mL of deionized water, mix fully.

   Solution 2: ZnSO4 Solution Dissolve 2.88 g of ZnSO4 ·7H2O with 8.64 mL of deionized water, mix fully.

   Solution 3: PB Solution Dissolve 6 g of Na2HPO4·12H2O and 0.5 g of NaH2PO4·2H2O with 300 mL of deionized water, mix fully.

   Solution 4: Liver Extracting Solution Dissolve 1 g of C2HCl3O2 with 100 mL of deionized water, mix fully.

   Solution 5: 1 M NaOH Solution Dissolve 4 g of NaOH with 100 mL of deionized water, mix fully.

   Solution 6: H3PO4 Solution Add 2 mL of Concentrated H3PO4 to 98 mL of deionized water, mix fully.

   Solution 7: Sample Solution 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 lower layer for analysis.

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

   3.2 Pretreatment of raw milk, reconstituted milk, finished milk sample:

   (1) Raw milk (powder): Weigh 1±0.01 g of raw milk into a 50 mL centrifuge tube. Add 8 mL of deionized water. Immediately oscillate for 30s, minx fully. Acidic samples such as yogurt: Weigh 1±0.01 g of homogenate egg into a 50 mL 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 ZnSO4 Solution((Solution 2) and add 100 µL of K4 [Fe (CN)6] Solution (Solution 1). Immediately oscillate for 30s, minx fully.

   (3) Add 1.8 mL of PB Solution (Solution 3). Centrifuge at 4000 r/min 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, minimum detection limit: 2 ppb

3.3 Pretreatment tissue (methond 1) sample:

(1) Weigh 1±0.01 g of homogenate and fish 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 oscillate for 60s, minx fully. Note: The tissue must be completely dispersed in the liquid

(2) Centrifuge at 4000 r/min for 10 min at room temperature.

(3) Take 20 µL for the supernatant analysis.

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

3.4 Pretreatment of tissue (methond 2, fish, shrimp, livestock) sample:

(1) Weigh 2±0.05 g of homogenate sample into a 50 mL centrifuge tube. Add 0.1 mL of H3PO4 Solution (Solution 6) and add 6 mL of Acetonitrile. Immediately oscillate for 2 min, minx fully.

(2) Centrifuge at 4000 r/min for 5 min at room temperature.

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

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

(5) Centrifuge at 4000 r/min 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, minimum detection limit: 2 ppb

3.5 Pretreatment of liver (chicken, swine) sample:

(1) Weigh 2±0.01 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 oscillate for 1 min, minx fully.

(2) Centrifuge at 4000 r/min for 5 min at room temperature.

(3) Take 1 mL of the intermediate layer solution to a new centrifuge tube. Add 20 µL of 1 M NaOH Solution (Solution 5), immediately oscillate for 30s. Centrifuge at 4000 r/min for 5 min at room temperature.

(4) Take 20 µL for analysis.

Note: Sample dilution factor: 4, minimum detection limit: 2 ppb

3.6 Pretreatment of feed sample:

(1) Weigh 1±0.01 g of homogenate sample into a 50 mL centrifuge tube. Add 10 mL of deionized water. Immediately oscillate for 60s, minx fully.

(2) Centrifuge at 4000 r/min for 10 min at room temperature.

(3) Take 20 µL for the supernatant analysis.

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

3.7 Pretreatment of serum, eggs sample:

(4) Weigh 1±0.05 g (1 mL) of homogenate sample into a 50 mL centrifuge tube. Add 1 mL of Methanol and 1 mL of Wash Buffer (Solution 8). Immediately oscillate for 30s, minx fully.

(5) Centrifuge at 4000 r/min for 5 min at room temperature.

(6) Take 20 µL for the supernatant analysis.

Note: Sample dilution factor: 3, minimum detection limit: 50 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.

(2) Add 0.1 mL of H3PO4 Solution (Solution 6) and add 5 mL of Acetonitrile. Immediately oscillate for 2 min, minx fully. Centrifuge at 4000 r/min 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 oscillate for 30s and 0.5 mL of Sample Solution (Solution 7). Oscillate for 30s

(5) Centrifuge at 4000 r/min 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, minimum detection limit: 3 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℃.

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 10s 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, 30s 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 15s 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 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/A0×100%

   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 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.

NOTE

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 kit to strong light.
5. Each reagent is optimized for use in the E-FS-E072. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other E-FS-E072 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. 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 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 °C.

Expiry date: expiration date is on the packing box.

PRODUCT USE LIMITATION

These products are intended for research use only.