Elabscience®

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SAs(Sulfonamides) ELISA Kit(0.5ppb)

Catalog No: E-FS-E037

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)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 be used to detect Sulfonamides (SAs) in samples, such as tissue, serum, honey, milk, urine, egg and other samples. This kit is composed of Micro ELISA Plate, HRP conjugate, antibody, standard and other supplementary reagents. The micro-plate provided in this kit has been pre-coated with SAs antigen. During the reaction, SAs in the samples or standard competes with SAs antigen coated on the solid phase supporter for sites of SAs antibody. Then Horseradish Peroxidase (HRP) conjugate is added to each micro 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

Sensitivity:0.5ppb (ng/mL)

Reaction mode:25℃,45 min~15min

Detection limit: Tissue (High detection limit method) ---0.5 ppb,

Tissue (Low detection limit method) ---2.5 ppb,

Egg, urine, serum---2 ppb, Honey---0.5 ppb, Milk---10 ppb, Water sample---1 ppb.

Cross-reactivity:

	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 (SDorSDZ)	308%	0.15
Sulfisomidine sodium(SM2')	241%	0.2
Sulfadimethoxypyrimidine(SDM)	175%	0.3
Sulfamethizole(SMT)	165%	0.3
Sulfaclozine(Esb3)	67%	0.8
Sulfathiazole(ST)	58%	0.9
Sulfachloropyridazine(SCPA)	58%	0.9
Sulfamethoxypyridazine(SMP)	57%	0.9
Sulfaquinoxaline(SQX)	42%	1
Sulfisoxazole(SIZ)	18%	3
Sulfamethoxazole(SMZ)	18%	3

Sample recovery rate: Tissue, honey, water sample---95%±25%. Urine, milk, serum---85%±25%. Egg---90%±25%.

Kits components

Item	Specifications
Micro ELISA Plate	96 wells
Standard Solution	1mL each (0ppb,0.025 ppb,0.075 ppb,0.225 ppb,0.675 ppb,2.025 ppb)
High Concentrated Standard(1 ppm) (red cap)	1 mL
HRP Conjugate(red cap)	5.5 mL
Antibody Working Solution(blue cap)	5.5 mL
Substrate Reagent A(white cap)	6 mL
Substrate Reagent B(black cap)	6 mL
Stop Solution(yellow cap)	6 mL
20×Concentrated Wash Buffer(white cap)	40mL
2×Redissolved Buffer(yellow cap)	50 mL
Product Description	1 copy

Other supplies required

Instruments: Microplatereader, Printer, Homogenizer, Oscillators, Centrifuge, Graduated pipette, Balance (sensibility 0.01g).

High-precision pipettor: Single-channel (20-200μL, 100-1000μL), Multi-channel(300μL).

Reagents: Ethyl acetate, N-hexane, Acetonitrile, Concentrated hydrochloric acid (HCl), $Na_2HPO_4 \cdot 12H_2O$, $Na_2HPO_4 \cdot 2H_2O$, Sodium hydroxide (NaOH).

Experimental preparation

Bring all reagents and samples to room temperature before use.

Open the microplate reader in advance (30 min), 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 1:0.2M NaOH

   Dissolve 0.8 g of sodium hydroxide with 100 mL deionized water.

   Solution2: 0.02M PB Buffer

   Dissolve 2.58 g $Na_2HPO_4 \cdot 12H_2O$ and 0.44g of $Na_2HPO_4 \cdot 3H_2O$with 500 mL deionized

water.

Solution 3:0.5M HCl

Add 4.3 mLConcentrated hydrochloric acid (HCl) to 100mLdeionized water, mixfully.

Solution 4: Re-dissolve solution (If sample is liquid, no need to dilute.)

Dilute 2×Concentrated Re-dissolvesolution to 1×Re-dissolvesolution with deionized water. It is used for re-dissolving the sample and can be stored at 4℃for 1 month.

3. Sample pretreatmentprocedure

3.1 Pretreatmentoftissuesample(High detection limit):

(1) Weigh3±0.05 g of homogenate into EP tube, add 3mLof Reagent 2(0.02M PB Buffer) and oscillate sufficiently. Then add 4mL of ethyl acetate and 2mL of acetonitrile. Oscillate for 10 min. Centrifuge at 4000 r/min for 10 min at room temperature.

(2) Remove 2mL of the supernatant(equal to 1g of sample) to another centrifuge tube, dry with Nitrogen Evaporators at 50-60℃.

(3) Dissolve the residue with 1 mL N-hexane, add 1mL of 1×Re-dissolvesolution and shake for 1 min to mix fully. Centrifuge for 5 min at 4000r/minat room temperature.

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

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

3.2 Pretreatment oftissue sample (Low detection limit):

(1) Weigh 3±0.05g of homogenate into EP tube, add 8mL of Reagent 2 (0.02M PB Buffer) and oscillate for 2 min, centrifuge at 4000 r/min for 10 min at room temperature.

(2) Take 50μL of the supernatant for analysis.

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

3.3 Pretreatmentof serum sample:

(1) Stand the samples at room temperaturefor 30 min and centrifuge for 10 min at 4000 r/min. Collect the supernatant and carry out the assay immediately. Tubes for blood collection should be disposable, non-pyrogenic, and non-endotoxin.

(2) Take 1 mL of serum into an EP tube, add 3 mL of Reagent 2(0.02M PB Buffer) and oscillate for 30 seconds.

(3) Take 50μL of the lower layer solution for analysis.

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

3.4 Pretreatmentof honey sample:

(1) Weigh1±0.05g of honey into a 50 mLEP tube, add 1mL of Reagent 3(0.5M HCl) and incubate at 37℃for 30 minutes.

(2) Add 2.5 mL of Reagent 1(0.2M NaOH, pH5.0) and 4 mL of ethyl acetate, oscillate for 5 min.Then centrifuge at 4000 r/min for 10 min at room temperature.

(3) Remove 2 mL of the supernatant to another centrifuge tube, dry with Nitrogen Evaporators at

50-60℃.

(4) Dissolvethe residue with 0.5mL of 1×Re-dissolvesolution, oscillate for 30 seconds.

(5) Take 50μLfor detection and analysis.

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

3.5 Pretreatmentof urine sample:

(1) Centrifuge the urine at 4000 r/min for 10min, collect the supernatant and carry out the assay.

(2) Add 3 mL of Reagent 2(0.02M PB Buffer) into1mLof urine, oscillate for 30 seconds.

(3) Take 50μL for detection and analysis.

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

3.6 Pretreatmentof milk sample:

(1) Dilute the milk with 0.02M PB Buffer for 20times (e.g.: add 20 μL of milk into 380 μL of 0.02M PB Buffer), oscillate for 30 s.

(2) Take 50μLfor detection and analysis.

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

3.7 Pretreatmentofwater sample:

(1) Take 200 μL of water sample into EP tube, add 200 μLof 2×Concentrated re-dissolvesolution and oscillate for 30 s.

(2) Take 50μL for detection and analysis.

Note: Sample dilution factor: 2, minimum detection dose: 1 ppb

3.8 Pretreatmentof eggs sample:

(1) Weigh2±0.05g of homogenate egg into a 50mL EP tube, add 8 mL of acetonitrile. Immediately oscillate for 10 min, centrifuge at 4000r/min for 15 min at 5℃.

(2) Remove 1mL of the supernatant to a 10 mL glass tube(clean and dry), dry the supernatant with Nitrogen Evaporators at 50-60℃.

(3) Dissolvetheresiduewith1mLofN-hexane and oscillate for 30 seconds. Add 1 mL of 1× Re-dissolvesolution and mix fullybyshaking for 1min. Centrifuge at 4000r/min for 5min at room temperature.

(4) Remove the hexane upper layer, and take 50μL of the lower layer for analysis.

Note: Sample dilution factor: 4, minimum detection dose: 0.2 ppb

Assay procedure

Centrifuge the sample again after thawing before the assay. Bring all reagents to room temperature for 30 min before use. **All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming.**Before experiment, dilute 40mL of concentrated wash buffer into 800mL wash working buffer with deionized or distilled water.

1. **Number:** Number the sample and standard in order (multiple well), and keep a record of standard

wells and sample wells.

2. **Add sample:** Add 50μL of Standard or Sample per well, then add 50μL of HRP conjugate to each well. Add 50μL of antibody working solution.Gently oscillate for 5s to mix thoroughly and cover the plate with sealer. Incubate for 45min at 25℃.
3. **Wash:** Uncover the sealer carefully, remove the liquid in each well. Immediately add 250μL of wash working buffer to each well and wash. Repeat the wash procedure for 5 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 50μL of substrate solution A to each well, and then add 50μL of substrate solution B. Gently oscillate for 5s to mix thoroughly. Incubate for 15min at 25℃ in the dark.
5. **Stop reaction:** Add 50μL of stop solution to each well, oscillate gently to mix thoroughly.
6. **OD Measurement:** Determine the optical density (OD value) of each wellat 450 nm with a microplate reader (the 450/630 nm double wavelength is recommended). This step should be finished in 10min after stop reaction.

Result analysis

1. **Absorbance(%)=A/A_0×100%**

   A: Average absorbance of standard or sample

   A_0: Average absorbance of 0ppb 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 software for accurate and fast analysis of batch samples.

Notes

1. The overall OD values will be lower when reagents have not been brought to room temperature before use or theroom 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 repeatability of this ELISA kit.
4. Micro ELISA plate should be covered with plate sealer.Prevent the reagents to strong light.
5. Do not useexpired kit and reagents of different batches.
6. TMB should be abandoned if it turns color. When OD value of standard(concentration: 0)＜0.5 unit(A_{450nm}＜0.5), it indicates the reagent isdeteriorated.
7. Stop solution is caustic, avoidcontact with skin and eyes.

Storage and valid period

Storage: Store at 2-8℃. Avoid freeze / thaw cycles.

Valid Period: 1 year, production date is on the packing box.