Elabscience®

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SAs (Sulfonamides) ELISA Kit

Catalog No: E-FS-E039

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 detect SAs in samples, such as tissue, serum, honey, milk, urine, etc. 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. During the reaction, SAs in the samples or standard competes with SAs 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 for color development. There is a negative correlation between the OD value of samples and the concentration of SAs. You can calculate the concentration of SAs in the samples by comparing the OD of the samples to the standard curve.

Technical indicator

Sensitivity:0.5ppb(ng/ml)

Reaction mode: 25℃, 45min~ 15min

Detection limit: Tissue (high detection limit)---0.5ppb

Tissue (low detection limit)---2.5ppb

Serum/urine---2ppb

Honey---0.5ppb

Milk---10ppb

Cross-reactivity:

Names	Cross-reativity	Sensitivity (ppb)
Sulfamethazine (SM2)	100%	0.5
Sulfamonomethoxine (SMM)	670%	0.07
sulfamerazine (SM1)	313%	0.15
Sulfadiazine (SD or SDZ)	308%	0.15
sulfamethoxine (SDM)	175%	0.3
Sulfamethizole (SMT)	165%	0.3
Sulfaquinoxaline (SQX）	42%	1

Sample recovery rate: Tissue/honey---95%±25%

Urine/milk/serum---85%±25%

Kits components

Item	Specifications
Micro ELISA Plate	96 wells
Standard Liquid	1mL each (0ppb, 0.5ppb, 1.5ppb, 4.5ppb, 13.5ppb, 40.5ppb)
High Standard (1 ppm) (red cap)	1mL
HRP Conjugate(red cap)	5.5mL
Antibody Working Solution(blue cap)	5.5mL
Substrate Reagent A(white cap)	6mL
Substrate Reagent B(black cap)	6mL
Stop Solution(yellow cap)	6mL
20×Concentrated Wash Buffer(white cap)	40mL
2×RedissolvedBuffer(yellow cap)	50mL
Product Description	1 copy

Other supplies required

Instruments: Microplate reader, Printer, Homogenizer, Nitrogen Evaporators, Oscillators, Centrifuge, Graduated pipette, Balance (sensibility 0.01g).

High-precision transferpettor: single channel(20-200μL, 100-1000μL), Multichannel(300μL).

Reagents: Ethyl acetate, n-hexane, dichloromethane, acetonitrile,$Na_2HPO_4 \cdot 12H_2O$, NaOH,concentrated HCl, $NaH_2PO_4 \cdot 2H_2O$.

Experimental preparation

Bring all reagents and samples to room temperature before use.

Open the micro-plate 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: 0.2M NaOH solution

Dissolve 0.8g NaOHto 100mL with deionized water

Solution 2: 0.02M PB buffer solution

Dissolve 2.58g $Na_2HPO_4 \cdot 12H_2O$ and 0.44g $NaH_2PO_4 \cdot 2H_2O$ to 500mL with deionized water

Solution 3: 0.5MHCl solution

Add 4.3mL concentrated HClinto 100mLdeionized water and mix thoroughly

Solution 4: Acetonitrile and dichloromethane mixed solution (Acetonitrile (V): dichloromethane (V)

= 1:1)

Solution 5: Redissolved Buffer

Double dilute the 2×Redissolved Bufferwith deionized water for the redissolutionof sample.

Redissolved Buffercan be stored for a month at 4℃

Solution 6: Washingsolution

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 oftissue (high detection limit) method I:

(1) Add 2±0.05g of homogeneous tissue sampleto a centrifuge tube, then add 8mlacetonitrile and dichloromethane mixed solution, oscillate for 2 min, centrifuge at above 4000r/min for 10min.
(2) Take 4mL of supernatant to the dry container, blow-dry in 50-60℃nitrogen or air.
(3) Dissolve the residual with 1mL n-hexane, add 1mL of redissolvedbuffer and oscillate strongfor 1min. Centrifuge at above 4000r/min for 5 min.
(4) Discard the upper n-hexane, take 50μLlower liquid foranalyze.

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

3.2 Pretreatment oftissue (high detection limit) method II:

(1) Add 3±0.05g of homogeneous tissue sample to a centrifuge tube, then add 3mlof 0.02M PB buffer solution, oscillate and mix thoroughly. Add 4ml ethyl acetate and 2ml acetonitrile, oscillate for 10 min, centrifuge at above 4000r/min for 10min.
(2) Take 2mL of upper liquid (about 1g of sample), blow-dry in 50-60℃nitrogen or air.
(3) Dissolve the residual with 1mL n-hexane, add 1mL of redissolved buffer and oscillate for 1min. Centrifuge at 4000r/min for 5 min.
(4) Discard the upper n-hexane, take 50uLlower liquid foranalyze.

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

3.3 Pretreatment of Tissue (low detection limit):

(1) Add 2.0±0.05g of homogeneous tissue sample to a centrifuge tube, then add 8mLof 0.02M PB buffer solution, oscillate for 2min,centrifuge at above 4000r/min for 10min.
(2) Take 50μL liquid for analyze.

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

3.4 Pretreatment of serum:

(1) Putblood sample atroom temperature for 30min, centrifuge at above 4000r/min for 10min, separate the serum or filter the serum.
(2) Take 1mL of serum, add 3mL of 0.02M PB buffer solution, mix for 30s.
(3) Take 50μL liquid for analyze.

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

3.5 Pretreatment of honey:

(1) Weigh1±0.05g of honey sample into 50mLa centrifuge tube, add 1mL of 0.5MHCl solution, put at 37℃ for 30min.

(2) Add 2.5mL of 0.2M NaOH solution (adjust the PH value to about 5), then add 4mL of ethyl acetate, oscillate for 5min, centrifuge at above 4000r/min at room temperature for 10min.

(3) Take 2mL of upper liquid, blow-dry in 50-60℃nitrogen. Add 0.5mL of dilutedredissolved buffertoredissolve, mix for 30s.

(4) Take 50μL liquid for analyze.

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

3.6 Pretreatment of urine:

(1) Mix3mLof 0.02M PB buffer solution and 1mL of centrifuged clear urine sample for 30s.

(2) Take 50μL liquid to analyze.

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

3.7 Pretreatment of milk:

(1) Dilute milk sample with 0.02M PB buffer solution with the ratio of 1:20(for example, 20μL milk + 380μL of 0.02M PB buffer solution), mix for 30s.

(2) Take 50μLliquid to analyze.

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

Assay procedure

Centrifuge the sample again after thawing before the assay. Bring all reagents to room temperature before use.**All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming.**Dilute 40mL of concentrated wash buffer into 800mLwash 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, then add 50μL of antibody working solution, cover the plate with sealer we provided, oscillate for 5s gently to mix thoroughly, incubate for 45min at 25℃.
3. **Wash:** uncover the sealer carefully, remove the liquid in each well. Immediately add 250μL of washing buffer to each well and wash. Repeat 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. **HRP conjugate:**add 100μL HRP conjugate to each well, shading light incubation for 30min at 25℃.
5. **Wash:** repeat step 3
6. **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 shading light for 15min at 25℃(The

reaction time can be extended according to the actual color change).

7. **Stop reaction:** add 50μL of stop solution to each well, oscillate gently to mix thoroughly.
8. **OD Measurement:** determine the optical density(OD value) of each well at 450 nm with a micro-plate 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 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 sampleto 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 on a large number of samples.

Notes

1. Overall OD value will be lower when reagents is not brought to room temperature before use or room temperature is below25℃.
2. During the washing procedure, if the wells turn dry, it will lead to bad linear standard curve and poor repeatability, move on to 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. Micro ELISA plate should be covered by plate sealer.Avoid the reagents to strong light.
5. Do not use expired kit and reagents of different batches of kits.
6. TMB should be abandoned if it turns color. When OD value of standard(concentration: 0)is below0.5 unit(A_{450nm}＜0.5), it indicates reagent is deteriorated.
7. Stop solution is caustic, avoid contact 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.