Elabscience®

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SAs(Sulfonamides) ELISA Kit

Catalog No: E-FS-E038

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, egg, 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, SA in the samples or standard competes with SA on the solid phase supporter for sites of SA 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. 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℃, 45min~ 15min

Detection limit: Tissue (high detection limit) ---0.5ppb,
Tissue (low detection limit) ---2.5ppb,
Serum, urine, egg---2ppb,
Honey---0.5ppb
Milk---10ppb

Cross-reactivity:

Names	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
sulfadimetine(SM2')	241%	0.2
sulfamethoxine(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

Sample recovery rate: Tissue, Honey---95%±25%
Urine,milk,serum,egg---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 ConcentratedStandard (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×Redissolved Buffer(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), Scroll instrument.

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

Reagents: Ethyl acetate, n-hexane, acetonitrile, NaOH, Na_2HPO_4•$12H_2O$, concentrated HCL,NaH_2PO_4•$2H_2O$.

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 0.8gNaOH with 100ml deionized water

Solution 2: 0.02M PB buffer

Dissolve 2.58g Na_2HPO4 • 12H2O and 0.44g NaH_2PO4 • 2H2O with 500 mL deionized water

Solution 3: 0.5M HCL.

Add4.3 mL concentrated HCL into 100 mLdeionized water and mix thoroughly.

Solution 4: Redissolved buffer

Double dilute the 2×Redissolve buffer with deionized water.

(2×Redissolved buffer (V): deionized water (V) = 1:1, Redissolved buffer could be kept for 1 month at 4℃).

Solution 5:Washing solution. Dilute 20×Concentrated Wash Buffer with deionized water 20×Concentrated Wash Buffer (V): Deionized water (V) = 1:19.

3. Sample pretreatmentprocedure

3.1 Pretreatment of tissue(High detection limit).

(1) Weigh 3±0.05ghomogeneous tissue samples and add them into centrifuge tube, add 3ml 0.02M PB buffer and mix sufficiently. Then add 4 mL ethyl acetate and 2 mLacetonitrile, oscillate 10 min, centrifuge at 4000r/min for 10 min.

(2) Pipette 2 mL supernatant liquid(about 1g sample), then dry in the air ordry with Nitrogen Evaporators at 50-60℃.

(3) Add 1mL n-hexane to dissolve the residual, then add 1mlredissolved buffer, oscillate strong for 1 min, centrifuge at 4000r/min for 5 min.

(4) Discard the upper n-hexane, take 50μLlower liquid for analyze.

Note: Sample dilution factor: 1; minimum detection dose: 0.5ppb.

3. 2 Pretreatment of tissue (Low detection limit):

(1) Weigh2.0±0.05ghomogeneous tissue sampleinto a centrifuge tube, add 8ml 0.02M PB buffer, oscillate 2 min, centrifuge at 4000r/min for 10 min.

(2) Take 50uL liquid foranalyze.

Note: Sample dilution factor:5; minimum detection dose: 2.5ppb.

3.3 Pretreatment of serum:

(1) Take bloodatroom temperature for30min, centrifuge at 4000r/min for 10 min, separate or filter serum.

(2) Take 1 mL serum, add 3ml 0.02 M PB and mix for 30s.

(3) Take 50μL serum sample for analysis.

Note: Sample dilution factor:4; minimum detection dose: 2ppb.

3.4Pretreatment of honey:

(1) Weigh1±0.05g honey sample into a centrifuge tube, then add 1ml 0.5M hydrochloric acid,

(2) Placethem at (37℃) for 30 min.

(3) Add 2.5mL 0.2M sodium hydroxide(ph=5),then add 4mL ethyl acetate, oscillate for 5 min, centrifuge at 4000r/min for 10 min.

(4) Pipette 2 mLsupernatant dry with Nitrogen Evaporators at 50-60℃. Add 0.5 mL diluted redissolvedbufferandmixfor 30s.

(5) Take 50μl liquid for analysis.

Note: Sample dilution factor:1; minimum detection dose:0.5ppb.

3.5 Pretreatment of Urine:

(1) Mix 3mL 0.02 M PB buffer and 1ml centrifuged clearurinefor 30s.

(2) Take 50μL liquid for analysis.

Note: Sample dilution factor:4;minimum detection dose:2ppb.

3.6 Pretreatment of Milk:

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

(2) Take 50μL liquid for analysis.

Note: Sample dilution factor:20; minimum detection dose:10ppb.

3.7 Pretreatment of egg:

(1) Mix egg white and yolkthoroughly with a homogenizer.

(2) Take 2.0±0.05ghomogenized egg samples to 50 mL centrifuge tubes, add 8ml acetonitrile, oscillate instantly for 10 min, centrifugehigher than 4000r/minfor 5 min at room temperature.

(3) Pipette 1ml supernatant to a 10 mL dry and clean tube,dry in the air or dry with nitrogen evaporators at 50-60℃.

(4) Add 1 mL n-hexane, whirl with a scroll instrument to dissolve dry residue, then add 1mlredissolved buffer and whirl for 1 min, centrifuge higher than 4000r/min for 5 min at room temperature.

(5) Discard upper layer organic phase liquid, take 50μL lower liquid for analysis.

Note: Sample dilution factor:4; minimum detection dose:2ppb.

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℃. (If the blue color is too shallow, can extend the incubation time properly.)
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 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 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 valueof 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.
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 reagent is deteriorated.
7. Stop solution is caustic, avoidcontact withskinandeyes.

Storage and valid period

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

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