Elabscience®

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Sulfonamides (SAs)ELISA Kit

Catalog No: E-FS-E040

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)
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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 Indirect-Competitive-ELISA as the method. It can detect Sulfonamides (SAs) in samples, such as tissue, serum, honey, milk and urine, etc. This kit is composed of Micro ELISA Plate, HRP conjugate, antibody, standard and other supplementary reagents. The microplate provided in this kit has been pre-coated with SAs antigen. 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. The concentration of SAs in the samples can be calculated by comparing the OD of the samples to the standard curve.

Technical indicator

Sensitivity: 0.1ppb(ng/mL)

Reaction mode: 25℃, 45min~15min

Detection limit: Tissue (high detection limit method)---0.1 ppb

Tissue (low detection limit method)---1 ppb

Serum, Urine, Egg---0.4 ppb

Honey---0.1 ppb

Milk---2 ppb

Feed---4ppb.

Cross-reactivity:

	Cross-reactivity	Sensitivity
Sulfamethoxazole(SMZ)	100%	0.1 ppb
Sulfamonomethoxine (SMM)	67%	0.15 ppb
Sulfadiazine (SD/SDZ)	33%	0.3 ppb

Sample recovery rate: Tissue, Honey, Egg---85±25%

Urine, Milk, Serum, Feed---85±25%

Kits components

Item	Specifications
Micro ELISA Plate	96 wells
Standard	1 mL each (0 ppb,0.1 ppb,0.3 ppb,0.9 ppb,2.7 ppb, 8.1 ppb)
High Standard (1ppm)(red cap)	1mL
HRPConjugate(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×Re-dissolve solution(yellow lid)	50mL
Manual	1 copy

Other supplies required

Instruments: Micro-plate 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: Acetic ether, N-hexane, Acetonitrile,$Na2HPO_4$•$12H_2O$,NaOH,Concentrated HCl,NaH_2PO_4•$2H_2O$.

Experimental preparation

1. **Notes for sample pretreatment:**

   Experimental apparatus should be clean and the pipette should be disposable to avoid cross-contamination during the experiment.

2. **Reagent preparation**

   Reagent 1: 0.1MPB buffer

   Dissolve 25.8 g Na_2HPO_4•$12H_2O$ and 4.4 g NaH_2PO_4•$2H_2O$ with1000 mL deionized water.

   Reagent 2:Acetonitrile-Acetic ether solution

   Mix 50 mL Acetonitrile and 50 mL Acetic ether fully.

   Reagent 3:0.5M HCl solution.

   Dilute 4.3mLConcentrated HCl with deionized water to 100 mL.

   Reagent 4: 0.2M NaOH

Dissolve0.8 g NaOHwith100 mL deionized water.

Reagent 5: Re-dissolve solution.

Dilute the 2×Re-dissolve solutionwith deionized water for re-dissolution of samples. The re-dissolve solution can be stable for 1 month at 4℃.

Reagent 6: Working wash buffer

Dilute the 20×Concentrated wash bufferwith deionized water (V/V=1:19).

3. Sample pretreatment

3.1. Pretreatment oftissue (high detection limit):

(1) Weigh2±0.05g of homogenatetissue sample into a centrifuge tube, add 1 mL of 0.1MPB buffer, oscillateuntil it mixed fully. Then add 7mL Acetonitrile-Acetic ether solution, oscillate fully for 2 min, centrifuge at a speed of over 4000r/min for 5 min at room temperature.

(2) Take 4mL of the upper layer liquid and dry with Nitrogen Evaporators at 50-60℃ or air.

(3) Add1mLn-hexane to dissolve the remaining dry material, then add 1mL re-dissolvesolution.Oscillate stronglyfor 30s and centrifuge at4000r/min for 5 min.

(4) Remove the upper layer n-hexane phase, take 50μL of the lower layer liquidfor analysis.

Sample dilution factor: 1,minimum detection dose: 0.1ppb.

3.2. Pretreatment oftissue (low detection limit)

(1) Weigh1±0.05g ofhomogenatetissue sample into a centrifuge tube,0.1MPB buffer,oscillate for 5 min. Centrifuge at a speed of over 4000r/min for 5min.

(2) Take 50μL for analysis.

Sample dilution factor: 10, minimum detection dose: 1ppb.

3.3 Pretreatment ofegg

(1) Homogenate the egg sample with homogenizer to mix the egg whites and yolks fully.

(2) Weigh2±0.05g of homogenateegg sample (mix 1 g egg powderand 3 mL deionized water, then take 2 mL, which equals to 2 g of fresh egg) into a centrifuge tube. Add 8 mLacetonitrile, then oscillatefully for 10 min immediately. Centrifuge at a speed of over 4000r/min for 5 min at room temperature.

(3) Take 1mL of the upper layer liquid and dry with Nitrogen Evaporators at 50-60℃ or air.

(4) Add1mLn-hexane to dissolve the remaining dry material, then add 1mL re-dissolvesolution.Oscillate stronglyfor 30s and centrifuge at4000r/min for 5 min.

(5) Remove the upper layer n-hexane phase, take 50μL of the lower layer liquidfor analysis.

Sample dilution factor: 4,minimum detection dose: 0.4ppb.

3.4 Pretreatment ofserum

(1) Place the serum sample at temperature for 30 min, then centrifuge at a speed of over 4000r/min for 10min. Separate out the serum or filter it.

(2) Take 1mL serum and add 3mL of 0.1M PB buffer, mix fully for 30 seconds.

(3) Take 50μL for analysis.

Sample dilution factor: 4, minimum detection dose: 0.4ppb.

3.5 Pretreatment of honey

(1) Weigh 1±0.05 g of honey sample into a 50mL centrifuge tube, add 1mL of 0.5M HCl solution, incubate for 30 min at 37℃.

(2) Add 2.5mL of 0.2M NaOH solution (the pH should be adjusted to about 5), then add 4mL acetic ether and oscillate for 5 min. Centrifuge at a speed of over 4000r/min for 10min at room temperature.

(3) Take 2mL of the upper layer liquid and dry with Nitrogen Evaporators at 50-60℃ or air. Add 0.5mL diluted re-dissolve solution, mix for 30 seconds.

(4) Take 50μL for analysis.

Sample dilution factor: 1, minimum detection dose: 0.1 ppb.

3.6 Pretreatmentof urine sample

(1) Mix 3mL of 0.1M PB buffer and 1 mL of centrifuged clear urine sample for 30 seconds.

(2) Take 50μL for analysis.

Sample dilution factor: 4, minimum detection dose: 0.4 ppb.

3.7 Pretreatmentof milk sample

(1) Dilute the milk sample with 0.1M PB buffer for 20 times (e.g., 20μL +380μL 0.02M PB buffer, V/V=1:19), mix for 30 seconds.

(2) Take 50μL for analysis.

Sample dilution factor: 20, minimum detection dose: 2 ppb.

3.8 Pretreatment of feed sample

(1) Weigh2±0.05g of feedsampleinto an EP centrifuge tube. Add 8 mLacetonitrile, then oscillatefully for 5 min immediately. Centrifuge at a speed of over 4000r/min for 5 min at room temperature.

4 Take 1mL of the upper layer liquid and dry with Nitrogen Evaporators at 50-60℃ or air.

5 Add1mLn-hexane to dissolve the remaining dry material and vortex mix for 30s, then add 1 mL0.1M PB buffer. Mix for 30s with vortex. Transfer the liquid into a 2 mL EP tubeand centrifuge at4000r/min for 5 min.

6 Remove the upper layer n-hexane phase, take 100μL of the lower layer liquid add 0.9mL0.1M PB buffer.Oscillatefor 1 minwith vortex.

7 Take 50μLfor analysis.

Sample dilution factor: 40,minimum detection dose: 4ppb.

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, Blank, or Sample per well, then add 50μL antibody working solution, cover the plate with sealer we provided, oscillate for 5s gently to mix thoroughly, shading light incubation for 45 min at25℃.
3. **Wash:**Uncover the sealer carefully, remove the liquid in each well. Immediately add 250μLof wash working 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 50μLof substrate solution A to each well, and then add 50μLof substrate solution B.Gently oscillate for 5s to mix thoroughly. Incubate shading light for 15min at 25℃.
7. **Stop reaction:**Add 50μLof stop solution to each well,gently oscillate and mix fully to stop the reaction.
8. **OD Measurement:**Determine the optical density (OD value) of each wellwith a micro-plate reader set to 450nm (the 450/630 nm double wavelength is recommended). This step should be finished in 10min after stop reaction.

Result analysis

1. **Absorbance% =** $A/A_0 \times 100\%$

   A: Average absorbance of standard solution or sample

   A_0: Average absorbance of 0 ppb Standard solution
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 the 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 on a large number of samples.

Notes

1. The overall OD values will be lower when reagents havenot been brought to room temperature before use or the room temperature <25℃.
2. During the washing procedure, if the wells turn dry, it will lead to bad linear standard curveand 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 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 may bedeteriorated.
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. The production date is on the packing box.

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