（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS！）

SM2(Sulfamethazine) ELISA Kit

Catalog No: E-FS-E043

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 Sulfamethazine (SM2) in tissues, honey and other samples. This kit is composed of Micro ELISA Plate pre-coated with coupled antigen, HRP conjugate, antibody, standard and other supplementary reagents. During the detection, after adding standard or sample solution, SM2 in the samples competes with pre-coated coupled antigen on the Micro ELISA Plate for SM2antibody.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 SM2. The residual quantity of SM2 in the samples can be calculated by comparing the OD of the samples to the standard curve.

Technical indicator

Sensitivity: 0.5 ppb(ng/mL)

Reaction mode: 25℃, 45min~ 15min

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

Tissue (low detection limit method) ---2.5 ppb.

Serum, Urine ---2 ppb. Honey---0.5 ppb. Milk ---10 ppb.

Cross-reactivity: Sulfamethazine ---100%.

Sample recovery rate: Tissue, Honey---95%±25%. Urine, Milk, Serum---85%±25%.

Kits components

Item	Specifications
Micro ELISA Plate	96 wells
Standard Solution	1mLeach (0ppb,0.5ppb,1.5ppb,4.5ppb,13.5ppb,40.5ppb.)
High ConcentratedStandard (1 ppm)	1 mL
HRPConjugate(Red lid)	5.5 mL
Antibody Working Solution(blue lid)	5.5mL
Substrate Reagent A(white lid)	6mL
Substrate Reagent B(black lid)	6mL
Stop Solution(yellow lid)	6mL
20×Concentrated Wash Buffer (white lid)	40mL
2×Re-dissolve solution(yellow lid)	50 mL
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), Multi-channel (300μL).

Reagents: Acetic ether, N-hexane, 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: 0.2MNaOH solution.

Dissolve0.8 g NaOHwith100mL deionized water.

Solution 2:0.02M PB buffer.

Dissolve 2.58 g $Na_2HPO_4 \cdot 12H_2O$ and 0.44 g $NaH_2PO_4 \cdot 2H_2O$ with500mL deionized water.

Solution 3:0.5M HCl solution.

Dilute 4.3mLConcentrated HCl with deionized water to 100 mL.

Solution 4: Re-dissolve solution.

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

3. Sample pretreatmentprocedure

3.1Pretreatment oftissue (high detection limit)

(1) Weigh3±0.05g of homogenatetissue sample into a centrifuge tube, add 3mL of 0.02MPB buffer, oscillateuntil it mixed fully. Then add 4mL acetic ether and 2 mL acetonitrile, oscillate fully for 10 min, centrifuge at a speed of over 4000r/min for 10min at room temperature.

(2) Take 2mLof the upper layer liquid (equal to 1 g sample) 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 1 min and centrifuge at4000r/min for 5 min.

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

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

3.2 Pretreatment oftissue (low detection limit)

(1) Weigh 2 ± 0.05g ofhomogenatetissue sample into a centrifuge tube,add 8mL of0.02M PB buffer,oscillate for 2 min. Centrifuge at a speed of over 4000r/min for 10min.

(2) Take 50μL for analysis.

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

3.3 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 0.02M PB buffer, mix fully for 30 seconds.

(3) Take 50μL for analysis.

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

3.4 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.

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

3.5 Pretreatmentof urine sample

(1) Mix 3mL of 0.02M PB buffer and 1 mL of centrifuged clear urine sample for 30 seconds.

(2) Take 50μL for analysis.

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

3.6 Pretreatmentof milk sample

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

(2) Take 50μL for analysis.

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

Assay procedure

Bring all reagents and samples to room temperature for more than 30 min before use. The washing solution may be crystallized when refrigerated, therefore it should be adjusted to room temperature to be fully dissolved. Each liquid must be shaken up before use. Take out the Micro plate and frame of needed amount, and keep the remained Micro plate into the valve bag and stored at 2-8℃.

Dilute the 20×Concentrated wash buffer to wash working buffer before the experiment.

1. **Number:**Number the sample and standard in order (multiplewell), 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$_0$×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 of sample 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 is below25℃.
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, production date is on the packing box.