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

Sulfamethoxazole(SMZ) ELISA Kit

Catalog No: E-FS-E021

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 Sulfamethoxazole(SMZ) in samples, such as tissue, serum, honey, fish milk and urine, etc.This kit is composed of Micro ELISA Plate, conjugate, antibody, standard and other supplementary reagents. The micro-plate provided in this kit has been pre-coated withSMZ. SMZ in the samples or standard competes with SMZ on the solid phase supporter for sites of SMZ 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 SMZ. The concentration of SMZ 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～15 min

Reaction rate: Sulfamethoxazole---100%

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

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

Honey---0.1 ppb, Serum, Urine, Egg---0.4 ppb, Milk---2 ppb, Fodder---4ppb

Sample recovery rate: Tissue/Honey/Egg ---85%±25%, Serum/Urine/Milk/Fodder---80%±25%

Kits components

Item	Specifications
ELISA Micro-plate	96 wells
Standard Liquid(black cap)	1mL each (0ppb, 0.1ppb, 0.3ppb, 0.9ppb, 2.7ppb, 8.1ppb)
High Concentrated Standard(1ppb)	1mL
HRPConjugate (Redcap)	5.5mL
Antibody Working Solution (bluecap)	5.5mL
Substrate Reagent A (whitecap)	6mL
Substrate Reagent B (Blackcap)	6mL
Stop Solution (Yellow cap)	6mL
20×Concentrated Wash Buffer (White cap)	40 mL
2×RedissolvedBuffer (yellow cap)	50mL
Product Description	1 copy

Other supplies required

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

Experimental preparation

1. **Sample pretreatment Notice:**experimental apparatus should be clean, and use disposable pipette tips to avoid cross-contamination during the experiment.

2. Solution preparation

Solution 1:0.2M NaOH. Dissolve 0.8g NaOH with 100 mL deionized water.

Solution 2: 0.5M HCl.Add 4.3mLConcentrated hydrochloric acid(HCl) to 100mL deionized water, mix fully.

Solution 3: 0.1M PBS buffer. Dissolve 25.8g $Na_2HPO_4 \cdot 12H_2O$ and 4.4 g $Na_2HPO_4 \cdot 2H_2O$with 1000mL deionized water.

Solution 4:Acetonitrile-ethyl acetate solution. Add 50 mL Acetonitrile and 50 mL Ethyl acetate to 100 mL glass bottle,mix fully.

Solution 5: Re-dissolveSolution. Dilute the 2×Concentrated Re-dissolveSolution with deionized water (1:1) for sample re-dissolvement.The solution can be stored at 4℃ for one month.

Solution 6: Working wash Buffer. Dilute the 20×Concentrated Wash Buffer with deionized water (1:19).

3. Sample pretreatment procedure

3.1 Pretreatmentof tissue sample (High detection limit, method 1):

(1) Weigh 2±0.05g of homogenate sample into 50 mL EP tube.Add 1mL of 0.1M PBS, Oscillate the sample into a paste with a vortex. Add 7mLAcetonitrile-ethyl acetate solution, Oscillate for 2min, centrifuge at 4000r/min for 10 min atroom temperature.

(2) Take 4 mL of theclean organic layer to a dry container, dry with nitrogen or air at 50-60℃.

(3) Re-dissolve the dry residual sediment with 1 mL of diluted re-dissolve solution. Add 1 mL of N-hexane and mix for 30 seconds. Centrifuge at a speed over4000r/min for 5 min atroom temperature.

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

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

3.2 Pretreatmentof tissue sample(Low detection limit):

(1) Weigh1±0.05g of homogenate into a 50 mL EP tube, add 9mL of 0.1M PBS Buffer and oscillate for 5min, centrifuge at a speed over 4000r/min for 5min at room temperature..

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

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

3.3 Pretreatmentof eggs sample

(1) Use homogenizer to homogenize egg sample,so that egg white and egg yolk fully mixed.

(2) Weigh2±0.05 g of homogenate sample into 50 mL EP tube. Add8mL of0.1M PBS Buffer and oscillate fully for 30s. Centrifuge at a speed over4000r/min for 5 min at room temperature.

(3) Take 1 mL of the supernatant to 10 mL clean dry glass, dry with nitrogen or airat50-60℃.

(4) Re-dissolve the dry residual sediment with 1 mL of diluted re-dissolve solution. Add 1 mL ofN-hexane and mix for 30 seconds. Oscillate for 1min, Centrifuge at a speed over 4000r/min for 5 min at room temperature. Discard the upper layer solution.

(5) Take 50 μLof the lower later solution for analysis.

Note: Sample dilution factor: 1

3.4 Pretreatmentof serum sample:

(1) Stand the serum for 30 min at room temperature. Centrifuge at a speed above 4000r/min for 10 min at room temperature, after the serum separated out.

(2) Take 1 mL of serum sample.Add 3 mL of 0.1M PBS Buffer and oscillate fully for 30s.

(3) Take 50 μL for analysis.

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

3.5 Pretreatmentof honey sample:

(1) Weigh1±0.05 g of honey sample into a50 mL EP tube. Add 1mLof 0.5M HCl. Incubate for 30 min at 37℃.

(2) Add 2.5mLof 0.2M NaOH and 4mLEthyl acetate.Oscillate for 5 min, centrifuge at a speed over4000r/min for 5 min at room temperature.

(3) Take 2mL of the upper layer solution to a dry container, dry with nitrogen or air at50-60℃.Re-dissolve the dry residual sediment with 0.5mL of diluted re-dissolve solution. Mix for 30s.

(4) Take 50μL for analysis.

Note: Sample dilution factor: 1

3.6 Pretreatment ofurine sample:

(1) Add 3mL of 0.1M PBS Buffer into 1mL of centrifuged clear urine sample, oscillate for 30seconds.

(2) Take 50μL for detection and analysis.

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

3.7 Pretreatmentof milk sample:

(1) Dilute100μLof milk with 0.1M PBS Buffer (1:19, v/v). Mix for 30 s.

(2) Take 50 μL for analysis.

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

3.8 Pretreatmentof feed sample:

(1) Weigh 2.0±0.05 g of feed sample into 50 mL polystyrene centrifuge tube, add 8ml acetonitrile, oscillate 5min, centrifuge at a speed over 4000 r/min for 5 min at room temperature.

(2) Take 1 mL of the clean organic layer to 10 mL clean dry glass, blow-dry in nitrogen or air with 50-60℃.

(3) Add1 mL N-hexane, use vortex to vortex sample for 30s, then add 1mL of 0.1MPB Buffer, vortex sample for 30s,transfer sample to 2mL polystyrene centrifuge tube, centrifuge at a speed over 4000 r/min for 5 min at room temperature.

(4) Remove the upper layer, take 50μL of the lower layer to 2mL EP tube, add 900uL of 0.1M PB Buffer, vortex sample for 1min, mixwell;

(5) Take 50 μL sample for analysis.

Note: Sample dilution factor: 40minimum detection dose: 4 ppb

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 wells), and keep a record of standard wells and sample wells.
2. **Add sample:** Add 50 μL of Standard, Blank, or Sample per well, then add 50 μL 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 250 μL of 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. **Color Development:**Add 50μL of substrate solution A to each well, and then add 50 μL of substrate solution B. Gently oscillate for 5s to mix thoroughly. Incubate shading lightfor 15min at 25℃ in the dark. (If the blue color is too shallow, can extend the incubation time properly.
5. **Stop reaction:** Add 50 μL of stop solution to each well, oscillate gently to mix thoroughly.
6. **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 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 valueof 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. Overall OD value will be lower when reagents is not brought to room temperature before use or room temperatureis 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)＜0.5 unit(A_{450nm}＜0.5), it indicates reagent is deteriorated.
7. Stop solution is caustic, avoid contact 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.