SMZ (Sulfamethoxazole) ELISA Kit

Catalog No: E-FS-E021

96T/96T*3

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.

Toll-free: 1-888-852-8623 Tel: 1-832-243-6086 Fax: 1-832-243-6017

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 Competitive-ELISA as the method for the quantitative detection. It can detect Sulfamethoxazole (SMZ) in samples, such as muscle, milk, honey, feed, etc. This kit is composed of ELISA Microtiter plate, HRP conjugate, antibody working solution, standard and other supplementary reagents. The microtiter plate in this kit has been pre-coated with coupled antigen. During the reaction, SMZ in the samples or standard competes with coupled antigen on the solid phase supporter for sites of anti-SMZ antibody. Then Horseradish Peroxidase (HRP) conjugate is added to each microtiter plate well, and substrate reagent 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

Reaction mode (Incubation time and temperature): 25℃; 45 min,15 min

Detection limit: Muscle (method 1) ---0.1 ppb; Muscle (method 2) ---1 ppb, Honey---0.1 ppb, Serum, Urine ---0.4 ppb; Milk---2 ppb; Feed---4 ppb; Egg ---0.2 ppb.

Cross-reactivity: Sulfamethoxazole---100%

Sample recovery rate: Muscle, Honey ---85%±25%, Serum, Urine, Milk, Feed---80%±25%

Kits components

Item	Specifications
ELISA Microtiter plate	96 wells
Standard Liquid	1 mL each (ppb=ng/mL=ng/g) (0 ppb, 0.1 ppb, 0.3 ppb, 0.9 ppb, 2.7 ppb, 8.1 ppb)
HRP Conjugate	5.5 mL
Antibody Working Solution	5.5 mL
Substrate Reagent A	6 mL
Substrate Reagent B	6 mL
Stop Solution	6 mL
20×Concentrated Wash Buffer	40 mL
2×Reconstitution Buffer	50 mL
Plate Sealer	3 pieces
Sealed Bag	1 piece
Manual	1 copy

Note: All reagent bottle caps must be tightened to prevent evaporation and microbial pollution.

Other materials required but not supplied

Instruments: Microplate reader, Printer, Homogenizer, Nitrogen evaporators, Water bath, Vortex mixer, Centrifuge, Graduated pipette, Balance (sensibility 0.01 g).

Micropipette: Single channel (20-200 μL, 100-1000 μL), Multichannel (30-300 μL).

Reagents: Ethyl acetate, Concentrated HCl, N-hexane, Acetonitrile, $Na_2HPO_4 \cdot 12H_2O$, NaOH $NaH_2PO_4 \cdot 2H_2O$.

Notes

1. The overall OD value will be lower when reagents have not been brought to room temperature before use or room temperature is below 25℃.
2. If the wells turn dry during the washing procedure, it will lead to bad linear standard curve and poor repeatability. Operate 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. FOR RESEARCH USE ONLY. ELISA Microtiter plate should be covered by plate sealer. Avoid th e kit to strong light.
5. **Each reagent is optimized for use in the E-FS-E021. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other E-FS-E021 with different lot numbers.**
6. Substrate Reagent should be abandoned if it turns blue color. When OD value of standard (concentration: 0)＜0.5 unit (A450nm＜0.5), it indicates the reagent may be deteriorated.
7. Stop solution is caustic, avoid contact with skin and eyes.
8. As the OD values of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique, washing technique or temperature effects), the operator should establish a standard curve for each test.
9. Even the same operator might get different results in two separate experiments. In order to get reproducible results, the operation of every step in the assay should be controlled.
10. **For mentioned sample fast and efficient extraction methods are included in the kit descriptio- n. Please consult technical support for the applicability if other sample need to be tested.**
11. The kit is used for rapid screening of actual samples. If the test result is positive, the instrument method such as HPLC, LC/MS, etc. can be used for quantitative confirmation.

Storage and expiry date

Store the kit at 2-8℃. Do not freeze any test kit components.

Return any unused microwells to their original foil bag and reseal them together with the desiccant provided and further store at 2-8℃.

Expiry date: expiration date is on the packing box.

Experimental preparation

Restore all reagents and samples to room temperature before use.

Open the microplate 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: 0.1 M PBS Buffer *(for muscle, serum, urine, milk, feed sample)*

   Dissolve 25.8 g of **$Na_2HPO_4 \cdot 12H_2O$** and 4.4 g of **$NaH_2PO_4 \cdot 2H_2O$** to 1000 mL with deionized water, mix fully.

   Solution 2: Acetonitrile-ethyl acetate Solution *(for muscle sample)*

   Add 50 mL of **Acetonitrile** and 50 mL of **Ethyl acetate** to 100 mL glass bottle, mix fully.

   Solution 3: 0.5 M HCl Solution *(for honey sample)*

   Add 4.3 mL of **Concentrated HCl** to 100mL with deionized water, mix fully.

   Solution 4: 0.2 M NaOH Solution *(for honey sample)*

   Dissolve 0.8 g of **NaOH** to 100 mL with deionized water, mix fully.

   Solution 5: Reconstitution Buffer *(for muscle, honey sample)*

   Dilute the **2×Reconstitution Buffer** with deionized water. (2×Reconstitution Buffer (V): Deionized water (V)=1:1) .The Reconstitution buffer can be store at 4℃ for a month.

   Solution 6: Wash Buffer

   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 of muscle (method 1) sample:

(1) Remove fat from sample, homogenize the sample with homogenizer.

(2) Weigh 2±0.05 g of homogenate muscle into 50 mL centrifuge tube. Add 1 mL of **0.1 M PBS Buffer** (Solution 1), vortex for 10 min. Add 7 mL of **Acetonitrile-ethyl acetate Solution** (Solution 2), Vortex for 4 min, centrifuge at 4000 r/min for 5 min at room temperature.

(3) Take 4 mL of the clean upper organic solution to a another centrifuge tube, dry at 50-60℃ of nitrogen evaporators or water bath.

(4) Redissolve the dry residual sediment with 1 mL of **N-hexane**. Add 1 mL of **Reconstitution Buffer (**Solution 5) and mix for 30s. Centrifuge at 4000 rpm for 5 min at room temperature.

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

Note: Sample dilution factor: 1, detection limit: 0.1 ppb

3.2 Pretreatment of muscle (method 2) sample:

(1) Remove fat from sample, homogenize the sample with homogenizer.

(2) Weigh 1±0.05 g of homogenate muscle into a 50 mL centrifuge tube, add 9 mL of **0.1 M PBS Buffer** (Solution 1) and vortex for 5 min, centrifuge at 4000 rpm for 5 min at room temperature..

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

Note: Sample dilution factor: 10, detection limit: 1 ppb

3.3 Pretreatment of serum (swine) sample:

(1) Stand the serum for 30 min at room temperature. Centrifuge at 4000 rpm for 10 min at room temperature.

(2) Take 1 mL of supernatant. Add 3 mL of **0.1 M PBS Buffer** (Solution 1) and vortex fully for 30s.

(3) Take 50 μL for analysis.

Note: Sample dilution factor: 4, detection limit: 0.4 ppb

3.4 Pretreatment of honey sample:

(1) For laboratory samples without crystallization, stir well. For the sample with crystallization phenomenon, place it in a closed water bath not exceeding 60℃, heat it, shake, stir after the sample is all melted, and cool to room temperature.

(2) Weigh 1±0.05 g of honey sample into a 50 mL centrifuge tube. Add 1 mL of **0.5 M HCl Solution** (Solution 3). Incubate at 37℃ for 30 min.

(3) Add 2.5 mL of **0.2 M NaOH Solution** (Solution 4) (adjust PH≈5), then add 4 mL of **Ethyl acetate**. Vortex for 5 min, centrifuge at 4000 rpm for 5 min at room temperature.

(4) Take 2 mL of the supernatant to a another centrifuge tube, dry at 50-60℃ of nitrogen evaporators or water bath.

(5) Redissolve the dry residual sediment with 0.5 mL of **Reconstitution Buffer (**Solution 5). Mix for 30s.

(6) Take 50 μL for analysis.

Note: Sample dilution factor: 1, detection limit: 0.1 ppb

3.5 Pretreatment of urine (swine) sample:

(1) Add 3 mL of **0.1 M PBS Buffer** (Solution 1) into 1 mL of centrifuged clear urine sample, vortex for 30s.

(2) Take 50 μL for detection and analysis.

Note: Sample dilution factor: 4, detection limit: 0.4 ppb

3.6 Pretreatment of milk sample:

(1) Dilute 100 μL of milk with **0.1 M PBS Buffer** (Solution 1) (100μL of milk +1.9 mL of 0.1M PBS Buffer). Mix for 30s.

(2) Take 50 μL for analysis.

Note: Sample dilution factor: 20, detection limit: 2 ppb

3.7 Pretreatment of feed sample:

(1) Homogenize the representative sample with a homogenizer and mix fully.

(2) Weigh 2±0.05 g of feed sample into 50 mL centrifuge tube, add 8 mL of **Acetonitrile**, vortex 5 min, centrifuge at a 4000 rpm for 5 min at room temperature.

(3) Take 1 mL of the upper organic layer to 10 mL clean dry glass tube, dry at 50-60℃ of nitrogen evaporators or water bath.

(4) Redissolve the dry residual sediment with 1 mL of **N-hexane**, Vortex for 30s, then add 1 mL of **0.1 M PBS Buffer** (Solution 1), Vortex sample for 30s. Centrifuge at 4000 rpm for 5 min at room temperature.

(5) Remove the upper organic layer, take 100 μL of the lower water layer to 2 mL centrifuge tube, add 900 μL of **0.1 M PBS Buffer** (Solution 1), vortex sample for 1 min, mix well;

(6) Take 50 μL sample for analysis.

Note: Sample dilution factor: 40, detection limit: 4 ppb

3.8 Pretreatment for egg sample:

(1) Use a homogenizer to homogenize the yolk and egg whites and mix them thoroughly.

(2) Weigh 2±0.05 g of homogenate sample into a 50 mL centrifugal tube, add 8 mL of **Acetonitrile** and vortex for 15 min. Centrifuge at 4000 rpm at room temperature for 10 min.

(3) Take 2 mL of supernatant into clean glass test tube (do not touch the fat layer) and dry at 50-60℃ with nitrogen evaporators or water bath.

(4) Dissolve the residue with 1 mL of **N-hexane**, and add 1 mL of **Reconstitution Buffer** (Solution 1), and mix thoroughly. Centrifuge at 4000 rpm at room temperature for 5 min.

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

Note: Sample dilution factor: 2, detection limit: 0.2 ppb

Assay procedure

Restore all reagents and samples to room temperature (25℃) before use. All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming. The unused ELISA Microtiter plate should be sealed as soon as possible and stored at 2-8℃.

1. **Number:** number the sample and standard in order (multiple wells), and keep a record of standard wells and sample wells. **Standard and Samples need test in duplicate.**
2. **Add Sample:** add 50 μL of **Standard or Sample** per well, then add 50 μL of **HRP Conjugate** to each well. Add 50 μL of **Antibody Working Solution**. Cover the plate with plate sealer, gently oscillate for 5s to mix thoroughly. Incubate at 25℃ for 45 min in shading light.
3. **Wash:** uncover the sealer carefully, remove the liquid in each well. Immediately add 300 μL of **Wash Buffer** (Solution 6) to each well and wash. Repeat wash procedure for 5 times, 30 sec 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 50 μL of **Substrate Reagent A** to each well, and then add 50 μL of **Substrate Reagent B**. Gently oscillate for 5s to mix thoroughly. Incubate at 25℃ for 15 min in shading light (The reaction time can be extended according to the actual color change).
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 (reference wavelength 630 nm) with a microplate reader. This step should be finished in 10 min after stop reaction.

Result analysis

1. **Absorbance (%) =A/A_0 ×100%**

   A: Average absorbance of standard or sample

   A0: 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 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 form for accurate and fast analysis on a large number of samples.

Sulfamethoxazole (E-FS-E021) **Standard Curve**