Technical Manual

SMZ (Sulfamethoxazole) ELISA Kit

- Catalogue Code: FSES0016
- Competitive ELISA Kit
- Research Use Only

Contents

1. Key features and Sample Types

Sensitivity:

0.1 ppb (ng/mL)

Assay Procedure:

25°C, 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

Cross Reactivity:

Sulfamethoxazole - 100%

Sample Recovery rate:

Muscle, Honey - 85%±25%, Serum, Urine, Milk, Feed - 80%±25%

Storage:

2-8°C for 6 months.

Expiry:

See Kit Label

2. Storage

Store the kit at 2~8°C. 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°C.

3. Test Principle

This kit uses a Competitive-ELISA method. 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.

4. Kit Contents

Each kit contains reagents for 96 assays including:

No.	Component	96-WellKit
1	ELISA Microtiter plate	96 wells
2	Standards	1 mL each (0ppb, 0.1ppb, 0.3ppb, 0.9ppb, 2.7ppb, 8.1ppb)
3	HRP Conjugate	5.5 mL
4	Antibody Working Solution	5.5 mL
5	Substrate Reagent A	6 mL
6	Substrate Reagent B	6 mL
7	Stop Solution	6 mL
8	20×Concentrated Wash Buffer	40 mL
9	2×Reconstitution Buffer	50 mL
10	Plate Sealer	3 pieces
11	Sealed Bag	1 piece
12	Manual	1 copy

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

Additional materials required:

- **Instruments:** Microplate reader, Printer, Homogenizer, Nitrogen evaporators, Water bath, Vortex mixer, Centrifuge, Graduated pipette, Balance (sensitivity 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 $NaH_2PO_4 \cdot 2H_2O$.

5. Experimental Preparation

Bring 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 pre-treatment 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. $Na_2HPO_4 \cdot 12H_2O$.

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°C 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 pre-treatment procedure

Targets may be distributed unevenly, resulting in no detection. To avoid this, ensure to take sufficient samples when sampling.

3.1 Pre-treatment of muscle (method 1) sample:

1. 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 the sample into a paste. Add 7 mL of **Acetonitrile-ethyl acetate Solution** (Solution 2), Vortex for 4min, centrifuge at 4000 r/min for 5 min at room temperature.
2. Take 4 mL of the clean upper organic solution to another centrifuge tube, dry at 50-60°C of nitrogen evaporators or water bath.
3. 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 r/min for 5 min at room temperature.
4. 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 Pre-treatment of muscle (method 2) sample:

1. 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 r/min for 5 min at room temperature.
2. Take 50 µL of the supernatant for analysis.
   Note: Sample dilution factor: 10, detection limit: 1 ppb

3.3 Pre-treatment of serum (swine) sample:

1. Stand the serum for 30 min at room temperature. Centrifuge at 4000 r/min 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 Pre-treatment of honey sample:

1. 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°C for 30 min.
2. Add 2.5 mL of **0.2 M NaOH Solution** (Solution 4) (adjust PH≈5), then add 4mL **Ethyl acetate**. Vortex for 5 min, centrifuge at 4000 r/min for 5 min at room temperature.
3. Take 2 mL of the supernatant to another centrifuge tube, dry at 50-60°C of nitrogen evaporators or water bath.
4. Redissolve the dry residual sediment with 0.5 mL of **Reconstitution Buffer** (Solution 5). Mix for 30s.
5. Take 50 µL for analysis.
   Note: Sample dilution factor: 1, detection limit: 0.1 ppb

3.5 Pre-treatment of urine (swine)* sample:

***Data validated in swine urine but pre-treatment can be applied for urine samples of multiple species.**

1. Add 3 mL of **0.1 M PBS Buffer** (Solution 1) into 1mL of centrifuged clear urine sample, vortex for 30s.
2. Take 50 µL for detection and analysis.
   Note: Sample dilution factor: 4, minimum detection limit: 0.4 ppb

3.6 Pre-treatment of milk sample:

1. Dilute 100 µL of milk with **0.1 M PBS Buffer** (Solution 1) (1:19, v/v, 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, minimum detection limit: 2 ppb

3.7 Pre-treatment of feed sample:

1. Weigh 2.0±0.05 g of feed sample into 50 mL centrifuge tube, add 8 mL of **Acetonitrile**, vortex 5 min, centrifuge at a 4000 r/min for 5 min at room temperature.
2. Take 1 mL of the upper organic layer to 10 mL clean dry glass tube, dry at 50-60°C of nitrogen evaporators or water bath.

3. 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 r/min for 5 min at room temperature.
4. 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.
5. Take 50 µL sample for analysis.
   Note: Sample dilution factor: 40, minimum detection limit: 4 ppb

6. Assay Procedure

Bring all reagents and samples to room temperature (25°C) 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°C.

1. **Number:** number the sample and standard in order (multiple wells), and keep a record of standard wells and sample wells. **Standard and Samples must be tested 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 vortex for 5s to mix thoroughly. Incubate at 25°C for 45 min away from direct sunlight.
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 absorbent paper (If bubbles exist in the wells, clean tips can be used to prick them).
4. **Colour Development:** add 50 µL of **Substrate Reagent A** to each well, and then add 50 µL of **Substrate Reagent B**. Gently vortex for 5s to mix thoroughly. Incubate at 25°C for 15 min away from direct sunlight (The reaction time can be extended according to the actual colour change).
5. **Stop Reaction:** add 50 µL of **Stop Solution** to each well, vortex 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.

7. Data Analysis

1 Absorbance (%) = $A/A_0 \times 100\%$

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 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.

8. 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°C.
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. ELISA Microplate should be covered by plate sealer. Avoid the kit to strong light.
5. **Each reagent is optimized for use in the FSES0016. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other FSES0016 with different lot numbers.**
6. Substrate Reagent should be abandoned if it turns blue colour. When OD value of standard (concentration: 0) < 0.5 unit (A450nm < 0.5), it indicates the reagents are 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. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
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.

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