Technical Manual

SAs (Sulfonamides of 3-in-1) ELISA Kit

- **Catalogue Code: FSES0028**
- **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; Milk - 2 ppb;
Serum, Urine, Eggs - 0.4 ppb; Honey - 0.1 ppb; Feed - 4 ppb.

Cross Reactivity:

Sulfamethoxazole (SMZ) - 100%; Sulfamonomethoxine (SMM) - 67%;

Sulfadiazine (SD/SDZ) - 33%

Sample Recovery rate:

Muscle, Honey, Eggs - 85±25%; Urine, Milk, Serum, 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 Sulfonamides of 3-in-1 (SAs) in samples, such as muscle, honey, milk and feed. 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, SA_S in the samples or standard competes with coupled antigen on the solid phase supporter for sites of anti-SA_S antibody. Then Horseradish Peroxidase (HRP) conjugate is added to each microtiter 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 SA_S. The concentration of SA_S 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 (0 ppb, 0.1 ppb, 0.3 ppb, 0.9 ppb, 2.7 ppb, 8.1 ppb)
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:

Other materials required but not supplied

- **Instruments:** Microplate reader, Printer, Homogenizer, Nitrogen evaporators, Water bath, Vortex mixer, Centrifuge, Graduated pipette, Balance (sensitivity 0.01g).
- **Micropipette:** Single channel (20-200 µL, 100-1000 µL), Multichannel (30-300 µL).
- **Reagents**: Ethyl acetate, N-hexane, Acetonitrile, $Na_2HPO_4 \cdot 12H_2O$, NaOH, Concentrated HCl, $NaH_2PO_4 \cdot 2H_2O$.

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

Please prepare solution according to the number of samples. Don't use up all components in the kit at once!

Solution 1: 0.1 M PB Buffer *(for muscle, serum, urine, milk, feed sample)*
Dissolve 25.8 g of **Na_2HPO_4•12H_2O** and 4.4 g of **NaH_2PO_4•2H_2O** to 1000 mL with deionized water.

Solution 2: Acetonitrile - Ethyl acetate Solution *(for muscle sample)*
Mix 50 mL of **Acetonitrile** and 50 mL of **Ethyl acetate** fully in glass bottle.

Solution 3: 0.5 M HCl Solution *(for honey sample)*
Dilute 4.3 mL of **Concentrated HCl** to 100 mL with deionized water.

Solution 4: 0.2 M NaOH Solution *(for honey sample)*
Dissolve 0.8 g of **NaOH** to 100 mL with deionized water.

Solution 5: Reconstitution Buffer *(for muscle, eggs, 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

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 sample into a centrifuge tube, add 1 mL of **0.1 M PB Buffer** (Solution 1), vortex and mixed fully. Then add 7 mL of **Acetonitrile - Ethyl acetate Solution** (Solution 2), vortex for 2 min, centrifuge at 4000 r/min for 5 min at room temperature.
2. Take 4 mL of the upper clear organic phase in another tube and dry at 50-60°C with nitrogen evaporators or water bath.
3. Dissolve the residue with 1 mL of **N-hexane**, then add 1 mL of **Reconstitution Buffer** (Solution 5). Vortex for 30s and centrifuge at 4000 r/min for 5 min.
4. Discard the N-hexane upper layer, take 50 µL of the lower layer for analysis.
Note: Sample dilution factor: 1, minimum detection limit: 0.1 ppb.

3.2 Pre-treatment of muscle (method 2) sample:

1. Weigh 1±0.05 g of homogenate muscle sample into a centrifuge tube, add 9 mL of **0.1 M PB Buffer** (Solution 1), vortex for 5 min. Centrifuge at 4000 r/min for 5 min.
2. Take 50 µL of clear liquid for analysis.
   Note: Sample dilution factor: 10, minimum detection limit: 1 ppb.

3.3 Pre-treatment of eggs sample:

1. Weigh 2±0.05 g of homogenate egg sample into a centrifuge tube. Add 8 mL of **Acetonitrile**, immediately vortex for 10 min, centrifuge at 4000 r/min for 5 min at room temperature.
2. Take 1 mL of the supernatant to a 10 mL glass tube (clean and dry), dry at 50-60°C with nitrogen evaporators or water bath.
3. Dissolve the residue with 1 mL of **N-hexane** and vortex for 30s. Add 1 mL of **Reconstitution Buffer** (Solution 5). Vortex for 1 min and centrifuge at 4000 r/min for 5 min.
4. Discard the upper organic phase, take 50 µL of the lower layer for analysis.
   Note: Sample dilution factor: 4, minimum detection limit: 0.4 ppb.

3.4 Pre-treatment of serum (swine)* sample:

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

1. Stand the blood samples at room temperature for 30 min and centrifuge for 10 min at 4000 r/min. Collect the supernatant which is serum sample. Tubes for blood collection should be disposable, non-pyrogenic, and non-endotoxin.
2. Take 1 mL of serum into centrifuge tube, add 3 mL of **0.1 M PB Buffer** (Solution 1) and vortex for 30s.
3. Take 50 µL of clear liquid for analysis.
   Note: Sample dilution factor: 4, minimum detection limit: 0.4 ppb.

3.5 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 the pH of the solution to about 5), then add 4 mL of **Ethyl acetate** and 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 with nitrogen evaporators
   or water bath. Dissolve the residue with 0.5 mL of **Reconstitution Buffer** (Solution 5) vortex for 30s.
4. Take 50 µL for analysis.

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

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

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

1. Centrifuge the urine at 4000 r/min for 10 min, collect the supernatant and carry out the assay.
2. Add 3 mL of **0.1 M PB Buffer** (Solution 1) into 1 mL of urine, vortex for 30s.
3. Take 50 µL for analysis

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

3.7 Pre-treatment of milk sample:

1. Dilute the milk sample with **0.1 M PB Buffer** (Solution 1) for 20 times (e.g., 100 µL of milk +1900 µL of 0.1 M PB Buffer, V/V=1:19), mix for 30s.
2. Take 50 µL for analysis.

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

3.8 Pre-treatment of feed sample:

1. Weigh 2±0.05 g of feed sample into a centrifuge tube. Add 8 mL of **Acetonitrile**, then vortex for 5 min immediately. Centrifuge at 4000 r/min for 5 min at room temperature.
2. Take 1 mL of the supernatant to another glass tube, dry at 50-60°C with nitrogen evaporators or water bath.
3. Add 1 mL of **N-hexane** to dissolve the residue and mix for 30s, then add 1 mL of **0.1 M PB Buffer** (Solution 1). Mix for 30s. Transfer the liquid into another centrifuge tube and centrifuge at 4000 r/min for 5 min.
4. Discard the upper organic phase, take 100 µL of the lower water layer liquid and add 900 µL of

   0.1 M PB Buffer (Solution 1). Vortex for 1 min.

5. Take 50 µL for analysis.

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

6. Assay Procedure

Bring all reagents and samples to room temperature 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 well), 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 50µL of **HRP Conjugate** to each well, then add 50 µL of **Antibody Working Solution**, cover the plate with plate sealer. Vortex for 5s gently 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, repeat wash procedure for 5 times, 30s 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 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 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 FSES0028. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other FSES0028 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.

Assay Genie 100% money-back guarantee!

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