SM_2 (Sulfamethazine) ELISA Kit

Catalog No: E-FS-E043

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 Sulfamethazine (SM_2) in samples, such as muscle, honey. 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, SM_2 in the samples or standard competes with coupled antigen on the solid phase supporter for sites of anti- SM_2 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 SM_2. The concentration of SM_2 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) ---0.5 ppb; Milk ---10 ppb; Muscle (method 2) ---2.5 ppb; Urine---2 ppb; Honey---0.5 ppb; Egg---1 ppb.

Cross-reactivity: Sulfamethazine ---100%.

Sample recovery rate: Muscle, Honey---95%±25%, Urine, Milk, Egg ---85%±25%.

Kits components

Item	Specifications
ELISA Microtiter plate	96 wells
Standard Liquid	1 mL each (ppb=ng/mL=ng/g) (0 ppb, 0.5 ppb, 1.5 ppb, 4.5 ppb, 13.5 ppb, 40.5 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, N-hexane, Acetonitrile, $Na_2HPO_4 \cdot 12H_2O$, NaOH, Concentrated HCl, $NaH_2PO_4 \cdot 2H_2O$.

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-E043. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other E-FS-E043 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 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 description. 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

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

Solution 1: 0.2 M NaOH Solution. *(for honey sample)*

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

Solution 2: 0.02 M PB Buffer. *(for muscle, swine urine , milk sample)*

Dissolve 2.58 g of **$Na_2HPO_4 \cdot 12H_2O$** and 0.44 g of **$NaH_2PO_4 \cdot 2H_2O$** to 500 mL with deionized water.

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

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

Solution 5: Wash Buffer

Dilute the **20×Concentrated Wash Buffer** with deionized water. (20×Concentrated Wash (V) Buffer: 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 3±0.05 g of homogenate muscle sample into a centrifuge tube, add 3 mL of **0.02 M PB Buffer** (Solution 2), vortex until it mixed fully. Then add 4 mL of **Ethyl acetate** and 2 mL of **Acetonitrile**, vortex fully for 10 min, centrifuge at 4000 r/min for 10 min at room temperature.

(3) Take 2 mL of the upper layer liquid and dry at 50-60℃ with nitrogen evaporators/water bath. (Please do it in a ventilated environment.)

(4) Add 1 mL each (ppb=ng/mL=ng/g)of **N-hexane** to dissolve the remaining dry material, then add 1 mL each (ppb=ng/mL=ng/g)of **Reconstitution Buffer** (Solution 4). Vortex strongly for 1 min and centrifuge at 4000 r/min for 5 min.

(5) Remove the upper layer **N-hexane** phase, take 50 μL of the lower layer liquid for analysis.

Note: Sample dilution factor: 1, detection limit: 0.5 ppb.

3.2 Pretreatment of muscle (method 2) sample:

(1) Weigh 2±0.05 g of homogenate muscle sample into a centrifuge tube, add 8 mL of **0.02 M PB Buffer** (Solution 2), vortex for 2 min. Centrifuge at 4000 r/min for 10 min.

(2) Take 50 μL for analysis.

Note: Sample dilution factor: 5, detection limit: 2.5 ppb.

3.3 Pretreatment of honey sample:

(1) Weigh 1±0.05 g of honey sample into a 50 mL centrifuge tube, add 1 mL each (ppb=ng/mL=ng/g)of **0.5 M HCl Solution** (Solution 3), incubate for 30 min at 37℃.

(2) Add 2.5 mL of **0.2 M NaOH Solution** (Solution 1) (the pH should be adjusted to about 5), then add 4 mL of **Ethyl acetate** and vortex for 5 min. Centrifuge at 4000 r/min for 10 min at room temperature.

(3) Take 2 mL of the upper layer liquid and dry at 50-60℃ with nitrogen evaporators/water bath. Add 0.5 mL of **Reconstitution Buffer** (Solution 4), mix for 30s.

(4) Take 50 μL for analysis.

Note: Sample dilution factor: 1, detection limit: 0.5 ppb.

3.4 Pretreatment of urine sample:

(1) Mix 3 mL of **0.02 M PB Buffer** (Solution 2) and 1 mL each (ppb=ng/mL=ng/g)of centrifuged clear urine sample for 30 s.

(2) Take 50 μL for analysis.

Note: Sample dilution factor: 4, detection limit: 2 ppb.

3.5 Pretreatment of milk sample:

(1) Dilute the milk sample with **0.02 M PB Buffer** (Solution 2) for 20 times (e.g: 20 μL+380 μL of 0.02 M PB Buffer), mix for 30 s.

(2) Take 50 μL for analysis.

Note: Sample dilution factor: 20, detection limit: 10 ppb.

3.6 Pretreatment of egg sample:

(1) Homogenize the sample with homogenizer.

(2) Weigh 2±0.05 g of homogenate sample into a 50 mL centrifuge tube, add 8 mL of **Acetonitrile**, vortex for 10 min. Centrifuge at 4000 r/min for 5 min at room temperature.

(3) Take 2 mL of the upper layer liquid into a 10 mL dry glass tube and dry at 50-60℃ with nitrogen evaporators/water bath.

(4) Add 1 mL each (ppb=ng/mL=ng/g)of **N-hexane,** vortex for 30 s to dissolve the remaining dry material, then add 1 mL each (ppb=ng/mL=ng/g)of **Reconstitution Buffer** (Solution 4). Vortex strongly for 1 min and centrifuge at 4000 r/min for 5 min.

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

Note: Sample dilution factor: 2, detection limit: 1 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 well), 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 50μL of **HRP Conjugate** to each well, then add 50 μL of **Antibody Working Solution**, cover the plate with plate sealer, oscillate for 5s gently to mix thoroughly, incubate for 45 min at 25℃ with shading light.
3. **Wash:** uncover the sealer carefully, remove the liquid in each well. Immediately add 300 μL of **Wash Buffer** (Solution 5) to each well and wash. Repeat wash procedure for 5 times, 30s intervals/time. Invert the plate and pat it against thick clean with 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 for 15 min at 25℃ with 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 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 form for accurate and fast analysis on a large number of samples.