SEM (Nitrofurazone) ELISA Kit

Catalog No: E-FS-E005

96T

Version Number: V1.1

Replace version: V1.1

Revision Date: 2026.02.09

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.

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Test principle

This kit uses Competitive-ELISA as the method for the quantitative detection. It can detect Nitrofurazone (SEM) in samples, such as honey, muscle, milk, feed, etc. This kit is composed of ELISA Microtiter plate, HRP conjugate, antibody working solution, standard and other supplementary reagents. The microtiter plate provided in this kit has been pre-coated with coupled antigen. During the reaction, SEM in the samples or standard competes with coupled antigen on the solid phase supporter for sites of anti- SEM 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 SEM. The concentration of SEM 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±2℃; 45 min, 15 min

Detection limit: Muscle, Liver, Honey, Milk, Egg ---0.1 ppb; Muscle (fish, shrimp) ---0.15 ppb;
Milk powder, Egg powder, Feed ---0.1 ppb

Cross-reactivity: SEM---100%, AMOZ, AOZ, AHD ---< 0.1%

Sample recovery rate: Muscle, Liver, Egg ---90%±15%, Honey, Milk ---80%±15%,
Milk powder, Egg powder, Feed ---85%±25%

Kits components

Item	Specifications
ELISA Microtiter plate	96 wells
Standard Liquid	1 mL each (ppb=ng/mL=ng/g) (0 ppb, 0.05 ppb, 0.15 ppb, 0.45 ppb, 1.35 ppb, 4.05 ppb)
Derivatization Reagent	10 mL
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	1 piece
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, NaOH, HCl, $K_2HPO_4 \cdot 3H_2O$, $ZnSO_4 \cdot 7H_2O$, Acetonitrile, Methanol, $Na_2Fe(CN)_5(NO) \cdot 2H_2O$.

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℃.
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 the kit to strong light.
5. **Each reagent is optimized for use in the E-FS-E005. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other E-FS-E005 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 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°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. After opening, the kit is stable for up to 1 month.

Expiry date: expiration date is on the packing box.

Experimental preparation

Restore all reagents and samples to room temperature before use.

Open the micro-plate 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: **0.36 M $Na_2Fe(CN)_5(NO)\cdot 2H_2O$ Solution** *(for milk, milk powder and egg powder sample)*

   Dissolve 10.7g of **$Na_2Fe(CN)_5(NO)\cdot 2H_2O$** to 100 mL with deionized water, mix fully.

   Solution 2: **1.04 M $ZnSO_4$ Solution** *(for milk, milk powder and egg powder sample)*

   Dissolve 29.8 g of **$ZnSO_4\bullet 7H_2O$** to 100 mL with deionized water, mix fully.

   Solution 3: **0.1 M K_2HPO_4 Solution**

   Dissolve 11.4 g of **$K_2HPO_4\bullet 3H_2O$** to 500 mL with deionized water, mix fully.

   Solution 4: **1 M HCl Solution**

   Dilute 8.6 mL of **HCl** to 100 mL with deionized water, mix fully.

   Solution 5: **1 M NaOH Solution**

   Dissolve 4 g of **NaOH** to 100 mL with deionized water.

   Solution 6: **Reconstitution Buffer**

   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 7: **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 milk sample:

(1) Take 5 mL of milk into centrifuge tube, add 250 µL of **0.36 M $Na_2Fe(CN)_5(NO) \cdot 2H_2O$ Solution** (Solution 1) and vortex for 30 s, then add 250 µL of **1.04 M $ZnSO_4$ Solution** (Solution 2) and vortex for 30 s, centrifuge at 4000 rpm for 10min at 15℃.

(2) Take 1.1 mL of supernatant to another centrifuge tube, add 4 mL of deionized water, 0.5 mL of **1 M HCl Solution** (Solution 4) and 100 µL of **Derivatization Reagent**, vortex for 5 min.

(3) Incubate overnight at 37℃(about 16 hours) or incubate in water bath at 50℃ for 3 hours (the effect of stratification will be affect when more than 50℃).

(4) Add 5 mL of **0.1 M K_2HPO_4 Solution** (Solution 3), 0.4 mL of **1 M NaOH Solution** (Solution 5) and 5 mL of **Ethyl acetate**, vortex for 5 min.

(5) Centrifuge at 4000 rpm at room temperature for 10 min.

(6) Take 2.5 mL of upper liquid to another tube, dry at 50-60℃ with nitrogen evaporators or water bath. (Please do it in a ventilated environment.)

(7) Dissolve the residual with 1 mL **N-hexane**, add 1 mL of **Reconstitution Buffer** (Solution 6) and vortex for 30 s. Centrifuge at 4000 rpm at room temperature for 10 min.

(8) Remove the upper layer n-hexane phase, take 50 µL lower liquid for analysis.

Note: Sample dilution factor: 2, detection limit: 0.1 ppb

3.2 Pretreatment of milk powder, egg powder sample:

(1) Weigh 1±0.05 g of sample into 50 mL centrifuge tube, add 4 mL of deionized water, 0.5 mL of **1 M HCl Solution** (Solution 4) and 100 µL of **Derivatization Reagent**, vortex for 5 min.

(2) Incubate overnight at 37℃(about 16 hours) or incubate with water bath at 50℃ for 3 hours (the effect of stratification will be affect when more than 50℃).

(4) Add 250 µL of **0.36 M $Na_2Fe(CN)_5(NO) \cdot 2H_2O$ Solution** (Solution 1), vortex for 30 s, then add 250 µL of **1.04 M $ZnSO_4$ Solution** (Solution 2), vortex for 30 s, centrifuge at 4000 rpm at 15℃ for 10 min.

(5) Take all supernatant to another centrifuge tube, add 5mL of **0.1 M K_2HPO_4 Solution** (Solution 3), 0.4 mL of **1 M NaOH Solution** (Solution 5) and 5 mL of **Ethyl acetate**, vortex for 5 min.

(6) Centrifuge at 4000 rpm at room temperature for 10 min.

(7) Take 2.5 mL of upper liquid to another tube, dry at 50-60℃ with nitrogen evaporators or water bath.

(8) Dissolve the residual with 1mL **N-hexane**, add 1 mL of **Reconstitution Buffer** (Solution 6) and vortex for 30 s. Centrifuge at 4000 rpm at room temperature for 10 min.

(9) Remove the upper layer n-hexane phase, take 50 µL lower liquid for analysis.

Note: Sample dilution factor: 2, detection limit: 0.1 ppb

3.3 Pretreatment of honey, muscle (livestock, fish, shrimp), liver, feed, egg sample:

(1) Remove fat from sample (except feed, honey and eggs). Homogenize the representative sample with a homogenizer and mix fully.

(2) Weigh 1±0.05 g of homogenate sample into 50 mL centrifuge tube, add 4 mL of deionized water, 0.5 mL of **1 M HCl Solution** (Solution 4) and 100 μL of **Derivatization Reagent**, vortex for 5min.

(3) Incubate overnight at 37℃ (about 16 hours) or incubate in water bath at 50℃ for 3 hours (the effect of stratification will be affect when more than 50℃).

(4) Add 5 mL of **0.1 M K_2HPO_4 Solution** (Solution 3), 0.4 mL of **1 M NaOH Solution** (Solution 5) and 5 mL of **Ethyl acetate**, vortex for 5 min.

(5) Centrifuge at 4000 rpm at room temperature for 10 min.

(6) Take 2.5 mL of upper liquid to another tube, dry at 50-60℃ with nitrogen evaporators or water bath.

(7) Dissolve the residual with 1 mL **N-hexane**, add 1 mL of **Reconstitution Buffer** (Solution 6) and vortex for 30 s. Centrifuge at 4000 rpm at room temperature for 10 min.

(8) Remove the upper layer n-hexane phase, take 50 μL lower liquid for analysis.

Note: Sample dilution factor: 2, detection limit: 0.1 ppb

detection limit of fish/shrimp: 0.15 ppb

Assay procedure

Restore 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℃.

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, then add 50 μL of **Antibody Working Solution**, cover the plate with plate sealer, gently oscillate for 5 s to mix thoroughly, incubate at 25±2℃ for 45 min in shading light.
3. **Wash:** uncover the sealer carefully, remove the liquid in each well. Immediately add 350 μL of **Wash Buffer** (Solution 7) to each well and wash. Repeat wash procedure for 5 times, 30 s 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 5 s to mix thoroughly. Incubate at 25±2℃ 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 (%) =$A/A_0 \times 100\%$**

   A: Average absorbance of standard or sample

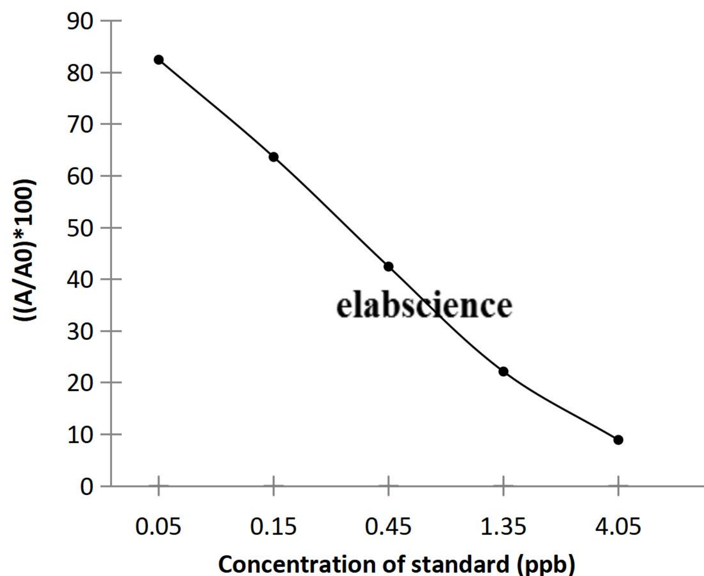
   A_0: Average absorbance of 0 ppb Standard

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.

Nitrofurazone (E-FS-E005) **Standard Curve**



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