Elabscience®

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SEM (Nitrofurazone Metabolite) ELISA Kit

Catalog No: E-FS-E004

96T

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.

Phone: 240-252-7368(USA) 240-252-7376(USA)
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 Indirect-Competitive-ELISA as the method. It can detect SEM in samples, such as honey, tissue, milk, fodder, etc. This kit is composed of Micro ELISA Plate, HRP conjugate, antibody, standard and other supplementary reagents. The micro-plate provided in this kit has been pre-coated with SEM. During the reaction, SEM in the samples or standard competes with SEM on the solid phase supporter for sites of SEM antibody. Then Horseradish Peroxidase (HRP) conjugate is added to each micro plate well, and TMB substrate is 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

Sensitivity: 0.05 ppb (ng/mL)

Reaction mode: 25℃, 45min~ 15min

Detection limit: Tissue,liver---0.1ppb,Honey/milk/casing---0.1ppb,
Milk powder/egg powder/fodder---0.1ppb, Fish and shrimp---0.15ppb

Cross-reactivity: Nitrofurazonemetabolite---100%, Furazolidone metabolite---＜0.1%,
Nitrofurantoin metabolite---＜0.1%, Furaltadone metabolite---＜0.1%

Sample recovery rate: Tissue/liver---90%±15%,Honey/milk/casing---80%±15%;
Milk powder/egg powder/fodder--85%±25%

Kits components

Item	Specifications
ELISA Micro-plate	96 wells
Standard Liquid	1mL each (0ppb,0.05ppb,0.15ppb,0.45ppb,1.35ppb,4.05ppb)
High Concentration Standard (100ppb)	1mL
DerivatizationReagent(black cap)	10mL
HRP Conjugate(red cap)	5.5mL
Antibody Working Solution(blue cap)	5.5mL
Substrate Reagent A(white cap)	6mL
Substrate Reagent B(black cap)	6mL
Stop Solution(yellow cap)	6mL
20×Concentrated Wash Buffer(white cap)	40mL
2×Reconstitution Buffer(yellow cap)	50mL
Product Description	1 copy

Other supplies required

Instruments: Micro-plate reader, Printer, Homogenizer, Nitrogen Evaporators, Oscillators, Centrifuge, Graduated pipette, Balance (sensibility 0.01g).

High-precision transferpettor: Single channel (20-200μL, 100-1000μL), Multichannel (300μL).

Reagents: Ethyl acetate, N-hexane, Sodium hydroxide, Concentrated HCl,K_2HPO_4•$3H_2O$,$K_2Fe(CN)_5(NO)$▪$2H_2O$,$ZnSO_4$•$7H_2O$.

Experimental preparation

Bring 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

Solution 1: 0.36M $K_2Fe(CN)_5(NO)$•$2H_2O$solution

Dissolve 11.9g$K_2Fe(CN)_5(NO)$•$2H_2O$ to 100mL with deionized water

Solution 2: 1.04M $ZnSO_4$•$7H_2O$solution (for milk and milk powder sample)

Dissolve 29.8g $ZnSO_4$•$7H_2O$ to 100mL with deionized water

Solution 3: 0.1M K_2HPO_4

Dissolve 11.4g K_2HPO_4•$3H_2O$ to 500mL with deionized water

Solution 4: 1 M HCl solution

Dilute 8.6mL concentrated HCl to 100mL with deionized water

Solution 5: 1 M NaOH solution

Dilute 4g NaOH to 100mL with deionized water

Solution 6:Reconstitutionsolution

Double dilute the 2× Reconstitution solution with deionized water,the solution can be stored at 4℃ for one month.

Solution 7: Wash buffer

Dilute the 20×Concentrated Wash Buffer with deionized water (1:19).

3. Sample pretreatmentprocedure

3.1 Pretreatment ofmilk (liquid):

(1) Take 5mL milk into 50mL EP tube, add 250 mLPotassium nitroprusside solution(Solution1) and oscillate for 30s, then add 250μL Zinc sulfatesolution(Reagent2) and oscillate for 30s, at last centrifuge at 4000r/min for 10min at 15℃;

(2) Take 1.1mL of supernatant, add 4mL of deionized water, 0.5mL of Solution 4 and 100uL of derivatizationreagent, oscillate for 5min.

(3) Incubate overnight(about 16h) at 37℃ or incubate with water bath(50℃) for 3h.

(4) Add 5mL of Solution 3, 0.4mL of Solution 5 and 5mL of ethyl acetate, oscillate for 5min.

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

(6) Take 2.5mL of upper liquid to another tube, blow-dry in nitrogen or air at 50-60℃.

(7) Dissolve the residual with 1mL n-hexane, add 1mL of reconstituted solution and oscillate for 30s. Centrifuge at 4000r/min at room temperature for 10 min.

(8) Discard the upper n-hexane, take 50uLlower liquid to analyze.

Note: Sample dilution factor: 2, minimum detection dose: 0.1ppb

3.2 Pretreatment of milk powder, egg powder:

(1) Weigh1±0.05g of sample into 50mL EP tube, add 4mL of deionized water, 0.5mL of Solution 4 and 100μL of derivatizationreagent, oscillate for 5min.

(2) Incubate overnight(about 16h) at 37℃ or incubate with water bath(50℃) for 3h.

(3)Add 250μLPotassium nitroprusside solution(Solution1) and oscillate for 30s, then add 250μL Zinc sulfatesolution(Solution2) and oscillate for 30s, at last centrifuge at 4000r/min for 10min at 15℃;

(4) Take the supernatant to another tube, add 5mL of solution 3, 0.4mL of solution 5 and 5mL of ethyl acetate, oscillate for 5min.

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

(6) Take 2.5mL of upper liquid to another tube, blow-dry in nitrogen or air at 50-60℃.

(7) Dissolve the residual with 1mL n-hexane, add 1mL of reconstituted solution and oscillate for 30s. Centrifuge at 4000r/min at room temperature for 10 min.

(8) Discard the upper n-hexane, take 50μLlower liquid to analyze.

3.3 Pretreatment ofhoney, tissue, casing, liver, fodder, egg:

(1) Weigh1±0.05g of sample into 50mL EP tube, add 4mL of deionized water, 0.5mL of Solution 4 and 100μL of derivatizationreagent, oscillate for 5min.

(2) Incubate overnight(about 16h) at 37℃ or incubate with water bath(50℃) for 3h.

(3) Add 5mL of Solution 3, 0.4mL of Solution 5 and 5mL of ethyl acetate, oscillate for 5min.

(4) Centrifuge at 4000r/min at room temperature for 10 min.

(5) Take 2.5mL of upper liquid to another tube, blow-dry in nitrogen or air at 50-60℃.

(6) Dissolve the residual with 1mL n-hexane, add 1mL of reconstituted solution and oscillate for 30s. Centrifuge at 4000r/min at room temperature for 10 min.

(7) Discard the upper n-hexane, take 50μLlower liquid to analyze.

3.4 Pretreatment of deli:

(1) Weigh1±0.05g of sample into 50mL EP tube, add 4.5mL of methyl alcohol and 0.5mL of deionized water, oscillate for 2min, Centrifuge at 4000r/min at room temperature for 5 min. Discard the liquid.

(2) Add 5mL of acetonitrile and 5mL of n-hexane, oscillate for 2min. Centrifuge at 4000r/min at room temperature for 5 min. Discard the liquid.

(3) Add 4mL of deionized water, 0.5mL of solution 4 and 100μL of derivatizationreagent, oscillate for 5min.

(4) Incubate overnight(about 16h) at 37℃ or incubate with water bath(50℃) for 3h.

(5) Add 5mL of solution 3, 0.4mL of Solution 5 and 5mL of ethyl acetate, oscillate for 5min.
(6) Centrifuge at 4000r/min at room temperature for 10 min.
(7) Take 2.5mL of upper liquid to another tube, blow-dry in nitrogen or air at 50-60℃.
(8) Dissolve the residual with 1mL n-hexane, add 1mL of reconstituted solution and oscillate for 30s.Centrifuge at 4000r/min at room temperature for 10 min.
(9) Discard the upper n-hexane, take 50uLlower liquid to analyze.

Assay procedure

Centrifuge the sample again after thawing before the assay. Bring all reagents to room temperature for 30 min before use. **All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming.**Dilute 40mL of concentrated wash buffer into 800mL wash working buffer with deionized or distilled waterbefore experiment.

1. **Number:** number the sample and standard in order (multiple wells), and keep a record of standard wells and sample wells.
2. **Add sample:** add 50μL of Standard, or Sample per well, then add 50μL of HRP conjugate to each well, then add 50μL of antibody working solution, cover the plate with sealer we provided, oscillate for 5s gently to mix thoroughly, incubate for 45min at 25℃.
3. **Wash:** uncover the sealer carefully, remove the liquid in each well. Immediately add 250μL of wash working buffer to each well and wash. Repeat wash procedure for 5 times, 30s 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. **HRP conjugate:**add 100μL HRP conjugate to each well, shading light incubation for 30min at 25℃.
5. **Wash:** repeat step 3
6. **Color Development:** add 50μL of substrate solution A to each well, and then add 50μL of substrate solution B. Gently oscillate for 5s to mix thoroughly. Incubate shading light for 15min at 25℃.(If the blue color is too shallow, can extend the incubation time properly.)
7. **Stop reaction:** add 50μL of stop solution to each well, oscillate gently to mix thoroughly.
8. **OD Measurement:** determine the optical density (OD value) of each well at 450 nm with a microplate reader (the 450/630 nm double wavelength is recommended). This step should be finished in 10min after stop reaction.

Result analysis

1 **Absorbance (%) =A/A_0×100%**

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 valueof 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 software for accurate and fast analysis on a large number of samples.

Notes

1. Overall OD value will be lower when reagents is not brought to room temperature before use or room temperature is below25℃.
2. During the washing procedure, if the wells turn dry, it will lead to bad linear standard curve and poor repeatability, move on to 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. Micro ELISA plate should be covered by plate sealer.Avoid the reagents to strong light.
5. Do not use expired kit and reagents of different batches of kits.
6. TMB should be abandoned if it turns color. When OD value of standard(concentration: 0)＜0.5 unit(A_{450nm}＜0.5), it indicates reagent is deteriorated.
7. Stop solution is caustic, avoid contact with skin and eyes.

Storage and valid period

Storage: Store at 2-8℃. Avoid freeze / thaw cycles.

Valid Period: 1 year, production date is on the packing box.