

AHD (Nitrofurantoin) ELISA Kit

Catalog No: E-FS-E004

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 Nitrofurantoin (AHD) in samples, such as muscle, honey, milk, 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, AHD in the samples or standard competes with coupled antigen on the solid phase supporter for sites of anti-AHD 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 AHD. The concentration of AHD 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°C; 45 min, 15 min.

Detection limit: Muscle (livestock), Liver, Honey, Milk ---0.04 ppb;

Milk powder, Feed, Egg powder, Egg---0.04 ppb; Muscle (fish, shrimp) ---0.06 ppb

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

Sample recovery rate: Muscle, Liver---85% ± 25%; Honey, Milk ---80% ± 20%;

Milk powder, Feed, Egg powder, Egg---85% ± 25%.

Kits components

Item	Specifications
ELISA Microtiter plate	96 wells
Standard Liquid	1 mL each (ppb=ng/mL=ng/g) (0 ppb,0.02 ppb,0.06 ppb,0.18 ppb,0.54 ppb,1.62 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	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, Vortex mixer, Nitrogen evaporators, Water bath, Centrifuge, Graduated pipette, Balance (sensitivity 0.01 g).

Micropipette: Single channel (20-200 µL, 100-1000 µL), Multichannel (30-300 µL).

Reagents: Ethyl acetate, N-hexane, NaOH, Concentrated HCl, K₂HPO₄•3H₂O, ZnSO₄•7H₂O, Na₂Fe (CN)₅NO •2H₂O.

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. 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-E004. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other E-FS-E004 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°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.

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 (30 min), 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 $\text{Na}_2\text{Fe}(\text{CN})_5\text{NO}$ Solution (*for milk, milk powder, egg powder sample*)

Dissolve 10.7 g of $\text{Na}_2\text{Fe}(\text{CN})_5\text{NO} \cdot 2\text{H}_2\text{O}$ to 100 mL with deionized water.

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

Dissolve 29.8 g of $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ to 100 mL with deionized water.

Solution 3: 0.1 M K_2HPO_4 Solution

Dissolve 11.4 g of $\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$ to 500 mL with deionized water.

Solution 4: 1 M HCl Solution

Dilute 8.6 mL of **Concentrated HCl** to 100 mL with deionized water.

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. ($2\times$ Reconstitution Buffer (V): Deionized water (V) = 1:1). The Reconstitution buffer can be store at 4°C for 1 month.

Solution 7: Wash Buffer

Dilute **20×Concentrated Wash Buffer** with deionized water. ($20\times$ 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 $\text{Na}_2\text{Fe}(\text{CN})_5\text{NO}$ 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 r/min for 10min at 15°C. (If a refrigerated centrifuge is not available, chill sample to approx 15°C prior to centrifugation.)
- (2) Take 1.1 mL of supernatant, 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°C (about 16 hours) or incubate in water bath at 50°C for 3 hours (the effect of stratification will be affect when more than 50°C).
- (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 r/min at room temperature for 10 min.
- (6) Take 2.5 mL of upper liquid to another tube, dry at 50-60°C 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 r/min at room temperature for 10 min.
- (8) Discard the upper n-hexane, take 50 μL lower liquid for analysis.

Note: Sample dilution factor: 2, detection limit: 0.04 ppb

3.2 Pretreatment of milk powder, egg powder sample:

- (1) Weigh 1 ± 0.05 g of sample into 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°C (about 16 hours) or incubate with water bath at 50°C for 3 hours (the effect of stratification will be affect when more than 50°C).
- (3) Add 250 μL of **0.36 M $\text{Na}_2\text{Fe}(\text{CN})_5\text{NO}$ 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 r/min at 15°C for 10 min.
- (4) Take all the supernatant to another tube, 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 r/min at room temperature for 10 min.
- (6) Take 2.5 mL of upper liquid to another tube, dry at 50-60°C 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 r/min at room temperature for 10 min.
- (8) Discard the upper n-hexane, take 50 μL lower liquid for analysis.

Note: Sample dilution factor: 2, detection limit: 0.04 ppb

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

- (1) Weigh 1 ± 0.05 g of homogenate sample into centrifuge tube, add 4 mL of deionized water, 0.5 mL of **1 M HCl Solution** (Solution 4) and 100 μ L of **Derivatization Reagent**, vortex for 5 min.
- (2) Incubate overnight at 37°C (about 16 hours) or incubate in water bath at 50°C for 3 hours (the effect of stratification will be affected when more than 50°C).
- (3) Add 5 mL of **0.1 M K₂HPO₄ Solution** (Solution 3), 0.4 mL of **1 M NaOH Solution** (Solution 5) and 5 mL of **Ethyl acetate**, vortex for 5 min.
- (4) Centrifuge at 4000 r/min at room temperature for 10 min.
- (5) Take 2.5 mL of upper liquid to another tube, dry at 50-60°C with nitrogen evaporators or water bath.
- (6) Dissolve the residual with 1 mL **N-hexane**, add 1 mL of **Reconstitution Buffer** (Solution 6) and oscillate for 30 s. Centrifuge at 4000 r/min at room temperature for 10 min.
- (7) Discard the upper n-hexane, take 50 μ L lower liquid for analysis.

Note: Sample dilution factor: 2, detection limit: 0.04 ppb
detection limit of fish, shrimp: 0.06 ppb

Assay procedure

Restore 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 well), and keep a record of standard wells and sample wells. **Standard and Samples need test in duplicate.**
2. **Add Sample:** add 50 μ L of **Standard or Sample** per well, then add 50 μ L of **HRP Conjugate** to each well, then add 50 μ L of **Antibody Working Solution**, cover the plate with plate sealer, oscillate for 5 s and mix thoroughly, incubate at 25°C for 45 min in shading light.
3. **Wash:** uncover the sealer carefully, remove the liquid in each well. Immediately add 300 μ L 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 μ L of **Substrate Reagent A** to each well, and then add 50 μ L of **Substrate Reagent B**. Gently oscillate for 5 s to mix thoroughly. Incubate at 25°C for 15 min in shading light (The reaction time can be extended according to the actual color change).
5. **Stop Reaction:** add 50 μ L 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.

Nitrofurantoin (E-FS-E004) Standard Curve

