Elabscience®

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AHD (Aminohydantoin Hydrochloride) 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 Aminohydantoin hydrochloride (AHD) in samples, such as honey, fish, shrimp, birds, liver and other samples. This kit is composed of Micro ELISA Plate, HRP conjugate, antibody, standard and other supplementary Solutions. The micro-plate provided in this kit has been pre-coated with AHD. During the reaction, AHD in the samples or standard competes with AHD on the solid phase supporter for sites of AHD 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 AHD. The concentration of AHD in the samples can be calculated by comparing the OD of the samples to the standard curve.

Technical indicator

Sensitivity: 0.02 ppb (ng/mL)

Reaction mode:25℃, 45min~15min

Detection limit: Tissue/liver---0.04ppb, Honey/milk/casing---0.04ppb,
Milk powder/egg/egg powder---0.04ppb
As fish/shrimp tissue are disturbed by some factor, detection limit is 0.06ppb

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

Sample recovery rate: Tissue/liver---85%±25%, Honey/milk/casing---80%±20%
Milk powder/ egg/egg powder---85%±25%

Kits components

Item	Specifications
ELISA Micro-plate	96 wells
Standard Liquid	1mL each (0ppb,0.02ppb,0.03ppb,0.18ppb,0.54ppb,1.62ppb)
High Concentrated Standard (100ppb)	1mL
DerivatizationSolution(black cap)	10mL
HRPConjugate(red cap)	5.5mL
Antibody Working Solution(blue cap)	5.5mL
Substrate Solution A(white cap)	6mL
Substrate Solution B(black cap)	6mL
Stop Solution(yellow cap)	6mL
20×Concentrated Wash Buffer(white cap)	40 mL
2×RedissolveBuffer(yellow cap)	50mL
Product Description	1 copy

Other supplies required

Instruments: Micro-plate reader, Printer, Homogenizer, 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,Concentratedhydrochloric acid, K_2HPO_4•$3H_2O$, Potassium nitroprusside($K_2Fe(CN)_5(NO)$·$2H_2O$), Glucuronidase, Zinc sulfate($ZnSO_4$•$7H_2O$).

Experimental preparation

Bring all Solutions and samples to room temperature before use.

Open the microplate reader in advance (30min), 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 Potassium nitroprussidesolution (milk, milk powder)

Dissolve 11.9g of Potassium nitroprusside to 100mL with deionized water

Solution2:1.04M Zinc sulfatesolution (milk, milk powder)

Dissolve 29.8g of Zinc sulfate to 100mL with deionized water

Solution3:0.1MK_2HPO_4

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

Solution4:1MHCl

Dilute 8.6mLof Concentratedhydrochloric acid to 100mL with deionized wate.

Solution 5: 1M NaOH

Dissolve 4g Sodium hydroxide to 100mL with deionized water

Solution6: Re-dissolvesolution (If sample is aquiform, no need dilute)

Dissolve 2×Re-dissolve solution to 1×Re-dissolve solution with deionized water for sample re-dissolution. Store at 4℃, valid for 1 month.

3. **Sample pretreatmentprocedure**

3.1 Pretreatmentofmilk (liquid):

(1) Take 5mL milk into 50mL EP tube, add 250μLof Potassium nitroprusside solution(Solution1) and oscillate for 30s, then add 250μLof Zinc sulfatesolution(Solution2) and oscillate for 30s, at last centrifuge at 4000r/min for 10min at 15℃;

(2) Removed 1.1mL of the supernatant to another centrifuge tube, add4mL deionized water, 0.5mLof HCl(1M)and 100μL of derivatizationSolution, oscillate for 3min;

(3) Incubate at 37℃(about 16h)for overnight, or water-bath at 50℃for 3h;

(4) Add5mL of K_2HPO_4(0.1M), 0.4mL of NaOH(1M) and 5mLethyl acetate, then oscillate for 5min;

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

(6) Removed 2.5mL of supernatant to another centrifuge tube, dry with Nitrogen Evaporators at

50-60℃;

(7) Dissolvedtheresiduewith1mLofn-hexane, add1mLof 1×Re-dissolve solutionand mix fullybyshakingfor 30 s;

(8) Centrifuge at 4000r/min for 5min at room temperature, take50μLfor detection and analysis.

Note: Sample dilution factor: 2,minimum detection dose: 0.04ppb

3.2 Pretreatmentof milk and egg powder:

(1) Weigh1±0.05g of homogenate milk into EP tube, add 4mL deionized water, 0.5mLHCl(1M) and 100μLof derivatizationsolution, oscillate for 5min;

(2) Incubate at 37℃(about 16h) for overnight, or water-bath at 50℃ for 3h;

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

(4) Removed all the supernatant to another centrifuge tube, add 5mL of K_2HPO_4 (0.1M), 0.4mL of NaOH(1M) and 5mLof ethyl acetate, then oscillate for 5min;

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

(6) Removed 2.5mL of supernatant to another centrifuge tube, dry with Nitrogen Evaporators at 50-60℃;

(7) Dissolvedtheresiduewith1mLofn-hexane, add1mL of1×Re-dissolve solutionand mix fullybyshakingfor30s;

(8) Centrifuge at 4000r/min for 5min at room temperature,take 50μLfor detection and analysis.

Note: Sample dilution factor:2,minimum detection dose: 0.04ppb

3.3 Pretreatmentofhoney, tissue, liver, casing, feed and egg:

(1) Weigh1±0.05g of homogenate milk into EP tube, add 4mL deionized water, 0.5mLof HCl(1M) and 100μLof derivatizationSolution, oscillate for 5min;

(2) Incubate at 37 ℃(about 16h) for overnight, or water-bath at 50 ℃ for 3h;

(4) Removeall the supernatant to another centrifuge tube, add 5mL of K_2HPO_4 (0.1M), 0.4mL of NaOH(1M) and 5mLof ethyl acetate, then oscillate for 5min;

(3) Centrifuge at 4000r/min for 10min at room temperature;

(4) Remove 2.5mL of supernatant to another centrifuge tube, dry with Nitrogen Evaporators at 50-60℃;

(5) Dissolvetheresiduewith1mLofn-hexane, add1mLof 1×Re-dissolve solution and mix fully byshakingfor 30s;

(6) Centrifuge at 4000r/min for 5min at room temperature, take50μLfor detection and analysis.

Note: Sample dilution factor:2,minimum detection dose: 0.04ppb

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 800mLwash working buffer with deionized or distilled water.

1. **Number:** number the sample and standard in order (multiplewell), 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℃.
7. **Stop reaction:**add 50μLof stop solution to each well,gently oscillate for 5s.
8. **OD Measurement:** determine the optical density (OD value) of each well at 450 nm with a micro-plate 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 sampleto 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 Solutions is not brought to room temperature before use or room temperatureis 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 Solutions to strong light.
5. Do not use expired kit and reagents of different batches.
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 Solution 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.