Elabscience®

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PHEA(Phenylethanolamine A) ELISA Kit

Catalog No:E-FS-E015

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 PHE A in samples, such as urine, tissue, 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 PHE A. During the reaction, PHE A in the samples or standard competes with PHE A on the solid phase supporter for sites of PHE A 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 PHE A. You can calculate the concentration of PHE A in the samples by comparing the OD of the samples to the standard curve.

Technical indicator

Sensitivity:0.1ppb(ng/mL)

Reaction mode: 25℃,30min～15min

Detection limit: Urine---0.1ppb,
Tissue---0.1ppb,
Fodder ---1ppb

Cross-reactivity: PHEA---100%,
Ketrintero---＜1%,
Albuterol---＜1%,
Ractopamine---＜1%

Sample recovery rate: Urine ---95%±15%,
Tissue/Fodder---85%±15%,

Kits components

Item	Specifications
Micro ELISA Plate	96 wells
Standard Liquid (Greencap)	1mL each (0ppb,0.1ppb,0.3ppb,0.9ppb,2.7ppb,8.1ppb)
High ConcentratedStandard (100ppb)	1mL
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
10×Redissolved Buffer(Yellow cap)	50mL
Manual	1 copy

Other supplies required

Instruments: Microplate 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: NaOH, Ethyl acetate, concentrated HCl,acetonitrile, n-hexane,Sodium sulfate,methyl alcohol.

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: 0.1M HCl solution

Dilute0.86mL concentrated HCl to 100mL with deionized water

Solution 2: 0.1M NaOH solution

Dissolve 0.4g NaOH to 100mL with deionized water

Solution 3: Acetonitrile-0.1M HCl solution

V(Acetonitrile)/V(0.1M HCl)=84:16

Solution 4: Redissolved Buffer

Dilute the 10×Redissolved Buffer with deionized water at dilution of 1:9,for sample re-dissolution.It can be stored at 4℃ for one month.

Solution 5: 1×Working washbuffer

Dilute the 20×Concentrated Wash Buffer with deionized water at dilution of 1:19.

3. **Sample pretreatment procedure**

3.1Pretreatment ofurine:

Take 50μL of clear urinesample to detect (the turbid urine sample should be filtered or centrifuge at 4000r/minfor 5min to get clear urine sample).Temporarily used samples should be kept frozento save.

Note: Sample dilution factor: 1, minimum detection dose: 0.1ppb

3.2 Pretreatment of tissue:

(1) Weigh2±0.05g of homogenatetissue sample,add 6mL of Acetonitrile-0.1M HCl solution, oscillate for 2min, and centrifuge at 4000r/min at room temperature for 10min.

(2) Take 3mL of the upper liquid, add 2mL of 0.1M NaOH solution,add 6mL of ethyl acetate,oscillate for 2min, and centrifuge at 4000r/min at room temperature for 10min. Take all the upper liquid to blow-dryat 50-60℃nitrogen or air.

(3) Add 1mL Redissolved Buffer, mix and oscillate for 30s, take 50uL liquid for analysis

Note: Sample dilution factor: 1, minimum detection dose: 0.1ppb

3.3 Pretreatment offodder:

(1) Weigh1.0±0.05g of homogenateFoddersample, add 10mL of methyl alcohol, then add 5gof Sodium sulfate, oscillate for 2min,and centrifuge at 4000r/min at room temperature for 10min.

(2) Take 1mL of upper liquid,blow-dryat 50-60℃nitrogen or air.Dissolve the driedresidual with 1mL Redissolved Buffer, add 1mL of n-hexane and mix for 30s. Centrifuge at 4000r/min at room temperature for 5min.

(3) Take 50μLlower liquid for analysis.

Note: Sample dilution factor: 10, minimum detection dose: 1ppb

Assay procedure

Centrifuge the sample again after thawing before the assay. Bring all reagents to room temperature 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, Blank, 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 30min 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 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.
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.