



Phenylethanolamine A ELISA Kit

(Catalog # E4751-100; 96 assays ; Storage at 4°C)

I. Introduction:

Phenylethanolamine is a trace amine with a structure similar to those of other trace phenethylamines as well as the catecholamine neurotransmitters. Phenylethanolamine A ELISA Kit is based on Indirect Competitive ELISA method. It can be used to detect PHE A in urine, tissue, feed samples. 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. The concentration of PHE A in the samples can be calculated by comparing the OD of the samples to the standard curve.

II. Applications:

- **Quantitative measurement of Phenylethanolamine A in Urine, Tissue, Feed samples.**
- **Sensitivity:** 0.1 ppb (ng/mL)
- **Sample recovery rate:** Urine - 95%±15%, Tissue/Feed - 85%±15%,
- **Cross-reactivity:** PHE A - 100%, Ketrintero - < 1%, Albuterol - < 1%, Ractopamine - < 1%
- **Detection limit:** Urine - 0.1 ppb, Tissue - 0.1 ppb, Feed - 1 ppb

III. Sample Type:

- Urine, Tissue, Feed

IV. Kit Contents:

Components	E4751-100	Part Number
ELISA Microplate	96 wells	E4751-100-1
Standard	1 ml x 6	E4751-100-2
High Concentrated Standard (100 ppb)	1 ml	E4751-100-3
HRP Conjugate	5.5 ml	E4751-100-4
Antibody Working Solution	5.5 ml	E4751-100-5
Substrate Reagent A	6 ml	E4751-100-6
Substrate Reagent B	6 ml	E4751-100-7
Stop Solution	6 ml	E4751-100-8
Wash Buffer (20x)	40 ml	E4751-100-9
Reconstitution Buffer (10x)	50 ml	E4751-100-10
Plate Sealer	3	E4751-100-11

V. User Supplied Reagents and Equipment:

- NaOH, Acetic ether, Concentrated HCl, Acetonitrile, Methanol, N-hexane, Anhydrous sodium sulfate.
- Microplate reader with 450 nm wavelength filter

VI. Storage Conditions and Reagent Preparation:

Please store the opened kit at 4°C, protect from light and moisture and use within 2 months.

0.1 M HCl Solution: Dilute 0.86 mL concentrated HCl to 100mL with deionized water

0.1 M NaOH Solution: Dissolve 0.4 g NaOH to 100mL with deionized water

Wash Buffer: Dilute the 20x Wash Buffer with deionized water. Mix 20x Concentrated Wash Buffer into Deionized water in the ratio of 1:19.

Reconstitution Buffer: Dilute the 10x Reconstitution Buffer with deionized water. The Reconstitution buffer can be store at 4°C for a month

Standard Concentration:

Standards	S1	S2	S3	S4	S5	S6
Concentration (ppb)	0	0.1	0.3	0.9	2.7	8.1

Sample pretreatment

Pretreatment of urine:

Take 50 µL of clear urine sample to detect (turbid urine sample should be filtered or centrifuge at 4000 r/min for 5 min to get clear sample). Keep the samples at -20°C for future use.

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



Pretreatment of tissue:

- (1) Weigh 2 ± 0.05 g of homogenate tissue sample; add 6mL of Acetonitrile- 0.1 M HCl solution, mix well for 2 min, and centrifuge at 4000 r/min at room temperature for 10 min.
- (2) Collect 3 mL of solution from the upper phase add 2 mL of 0.1 M NaOH solution, add 6 mL of ethyl acetate, mix well for 2 min, and centrifuge at 4000 r/min at room temperature for 10 min. Take all the upper liquid to blow-dry at 50-60°C with nitrogen or Water bath.
- (3) Add 1 mL of Reconstitution buffer, mix and shake for 30 sec. Take 50 μ L liquid for analysis

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

Pretreatment of feed:

- (1) Weigh 1.0 ± 0.05 g of homogenate Feed sample, add 10 mL of methyl alcohol, then add 5 g of Anhydrous sodium sulfate, oscillate for 2 min, and centrifuge at 4000 r/min at room temperature for 10min.
- (2) Take 1 mL of upper liquid, blow-dry at 50-60°C with nitrogen or Water bath. Dissolve the dried residual with 1 mL of Reconstitution buffer, add 1 mL of n-hexane and mix for 30 sec. Centrifuge at 4000 r/min at room temperature for 5 min.
- (3) Take 50 μ L of lower phase liquid for analysis.

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

VII. Assay Protocol:

Note: Restore all reagents and samples to room temperature before use. All the reagents should be mixed thoroughly by gentle mixing before pipetting. Avoid bubble formation.

It is recommended that all standards and samples be run at least in duplicate. A standard curve must be run with each assay.

1. Add 50 μ L of **standard or sample** per well, then add 50 μ L of **HRP conjugate** and 50 μ L of **antibody working solution**, cover the plate sealer, shake for 5 sec gently to mix thoroughly, incubate for 30 min at 25°C.
2. Remove the sealer carefully. Aspirate the liquid. Immediately add 300 μ L of **wash buffer (1x)** to each well and wash. Repeat wash procedure for 5 times, 30 s intervals each time. Invert the plate and pat it against thick clean absorbent paper (If bubbles exist in the wells, clean tips can be used to remove them).
3. Add 50 μ L of **Substrate reagent A** to each well, and then add 50 μ L of **Substrate reagent B**. Gently mix to mix thoroughly. Incubate at 25°C for 15 min in dark. Note: the reaction time can be shortened or extended according to the actual color change, but not more than 30 min.
4. Add 50 μ L of **stop solution** to each well, shake the plate gently to mix thoroughly.
5. Determine the optical density (OD value) of each well at 450 nm (reference wavelength 630 nm) with a microplate reader. Read the plate within 10 min after adding stop solution.

Calculation:

$$\text{Absorbance (\%)} = A/A_0 \times 100\%$$

A: Average absorbance of standard or sample

A0: Average absorbance of 0 ppb Standard

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.

VIII. Related Products:

- Enrofloxacin (ENR) ELISA Kit (E4277)
- Diethylstilbestrol (DES) ELISA Kit (E4278)
- Ciprofloxacin (Cipro) ELISA Kit (E4365)

FOR RESEARCH USE ONLY! Not to be used on humans.