（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K846-M

Specification：96T (40 samples)

Measuring instrument：Microplate reader(430-470 nm)

Detection range：4.25-500.00 μmol/L

Elabscience® Phenylalanine (Phe) Colorimetric Assay Kit

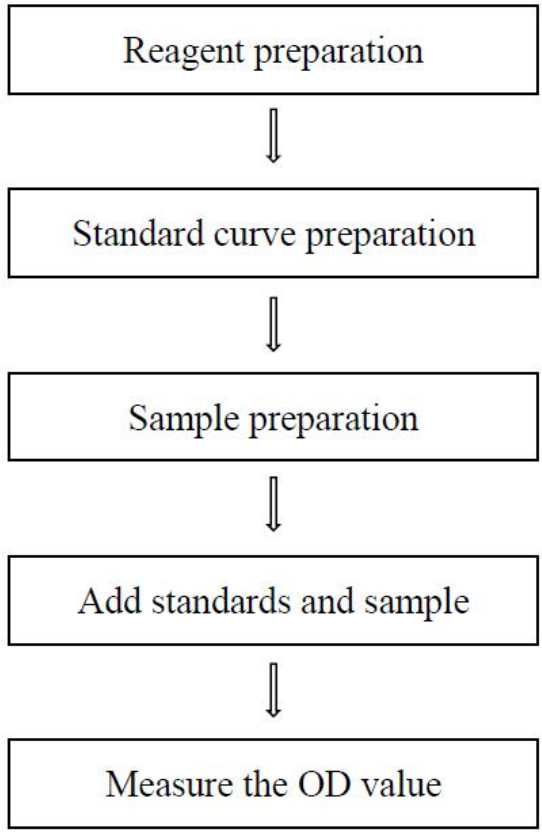
This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 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.

Table of contents

Assay summary



Intended use

This kit can be used to measure phenylalanine (Phe) content in serum (plasma), urine, animal (plant) tissue, cell and cell culture supernatant samples.

Detection principle

Phenylalanine (Phe), chemically known as 2-amino-3-phenylpropionic acid, is an essential amino acid involved in a variety of biosynthetic processes. In the body, most Phe is oxidized to tyrosine by phenylalanine hydroxylase, and together with tyrosine, it synthefies important neurotransmitters and hormones, and participates in glucose metabolism and fat metabolism. Phenylalanine is catalyzed by enzyme , and the chromogenic agent has an absorption peak at 450 nm. The Phe content of the sample can be calculated by measuring the OD value of the chromogenic substance at 450 nm.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer Solution	13 mL × 1 vial	-20℃, 12 months, shading light
Reagent 2	Enzyme Reagent	0.11 mL × 1 vial	-20℃, 12 months, shading light
Reagent 3	Accelerant	Powder × 4 vials	-20℃, 12 months, shading light
Reagent 4	Substrate	Powder × 2 vials	-20℃, 12 months, shading light
Reagent 5	Chromogenic Solution	2.4 mL × 1 vial	-20℃, 12 months, shading light
Reagent 6	10 mmol/L Standard Solution	0.24 mL × 1 vial	-20℃, 12 months, shading light
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Microplate reader (430-470 nm, optimum wavelength: 450 nm), Vortex mixer

Reagents:

Normal saline (0.9% NaCl)

Consumptive material:

10kDa MWCO Spin Filter

Reagent preparation

① Equilibrate all the reagents to 25°C before use.

② The preparation of control working solution:

Dissolve one vial of accelerant with 3 mL of buffer solution, mix well to dissolve. The reaction working solution should be prepared on spot and use it up in the same day.

③ The preparation of measuring working solution:

Dissolve one vial of substrate with 3 mL of control working solution, mix well to dissolve. The measuring working solution should be prepared on spot and use it up in the same day.

④ The preparation of 500 μmol/L standard solution:

Before testing, please prepare sufficient 500 μmol/L standard solution. For example, prepare 1000 μL of 500 μmol/L standard solution (mix well 50 μL of 10 mmol/L standard solution and 950 μL of double distilled water). Store at 2-8°C for a week protected from light.

⑤ The preparation of standard curve:

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 500 μmol/L standard with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 100, 200, 250, 300, 350, 400,

500 μmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**100**	**200**	**250**	**300**	**350**	**400**	**500**
500 μmol/L standard (μL)	0	40	80	100	120	140	160	200
Double distilled water (μL)	200	160	120	100	80	60	40	0

Sample preparation

① Sample preparation

Liquid sample: Add liquid sample into 10 kDa MWCO Spin Filter and centrifuge at 12000×g for 15 min, collect the filtrate. Mix well 200μL filtrate and 1 μL enzyme reagent, and stand at 25℃ for 10min. Keep it on ice for detection.

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 100 mg).

② Wash tissue in cold normal saline (0.9% NaCl).

③ Homogenize 100 mg tissue in 900 μL normal saline (0.9% NaCl) with a dounce homogenizer at 4°C.

④ Centrifuge at 10000×g for 10 min at 4℃ to remove insoluble material.

⑤ Collect supernatant and add it to 10kDa MWCO Spin Filter. Centrifuge at 12000×g for 15 min at 4℃.

⑥ Collect the filtrate and preserve it on ice for detection. For every 200 μL filtrate sample, add 1 μL of enzyme reagent and stand at 25°C for 10 min, keep it on ice for detection.

Cell (adherent or suspension) samples:

① Harvest the number of cells needed for each assay (initial recommendation 1×10^6 cells).

② Homogenize 1×10^6 cells in 200 μL normal saline (0.9% NaCl) with a dounce homogenizer at 4°C.

③ Centrifuge at 10000×g for 10 min at 4℃ to remove insoluble material.

④ Collect supernatant and add it to 10kDa MWCO Spin Filter. Centrifuge at 12000×g for 15 min at 4℃.

⑤ Collect the filtrate and preserve it on ice for detection. Mix well 200μL filtrate and 1 μL enzyme reagent, and stand at 25℃ for 10min. Keep it on ice for detection.

② **Dilution of sample**

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
10% Mouse liver tissue homogenate	2-4
10% Mouse stomach tissue homogenate	2-3
10% Peach flesh tissue homogenate	1
Rat plasma	2-3
Human serum	2-4
Rabbit serum	2-3
1×10^6 Jurka cells	1
1×10^6 Mouse spleen cells	1
Jurka cell culture supernatant	2-4
Mouse spleen cell culture supernatant	2-4

Note: The diluent is normal saline (0.9% NaCl). For the dilution of other sample types, please do pretest to confirm the dilution factor.

Operating steps

① Standard well: Add 20 μL of different concentrations solution to standard well.

Sample well: Add 20 μL of sample to sample well.

Control well: Add 20 μL of sample to control well.

② Add 20 μL of chromogenic solution to each well.

③ Add 100 μL of measuring working solution to standard and sample wells.

④ Add 100 μL of control working solution to control wells.

⑤ Mix fully with microplate reader for 5 s. Incubated at 37°C for 30 min, measure the OD value of each well at 450 nm with microplate reader.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard #①) from all standard readings. This is the absoluted OD value.

3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

1. Liquid sample:

$$\text{Phe content (μmol/L)} = \frac{\Delta A - b}{a} \times f$$

2. Tissue sample:

$$\text{Phe content (μmol/kg wet weight)} = \frac{\Delta A - b}{a} \div \frac{m}{V} \times f$$

3. Cell sample:

$$\text{Phe content (nmol/10^6)} = \frac{\Delta A - b}{a} \div \frac{n}{V} \times f$$

[Note]

ΔA: $\Delta A = OD_{sample} - OD_{control}$.

m: The wet weight of sample, g.

n: The number of cell samples, 10^6.

v: The volume of normal saline (0.9% NaCl) in the preparation step, mL.

f: Dilution factor of sample before test.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	175.00	275.00	325.00
%CV	1.6	0.9	1.2

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	175.00	275.00	325.00
%CV	2.4	3.3	4.0

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 95.7%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (μmol/L)	175	275	325
Observed Conc. (μmol/L)	166.3	261.3	315.3
recovery rate(%)	95	95	97

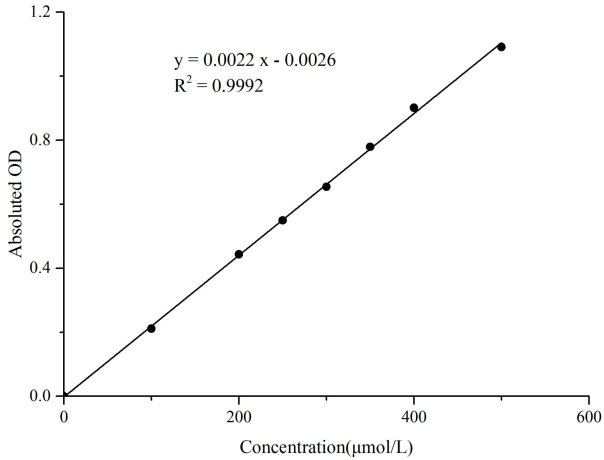
Sensitivity

The analytical sensitivity of the assay is 4.25 μmol/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve:

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only:

Concentration (μmol/L)	**0**	**100**	**200**	**250**	**300**	**350**	**400**	**500**
OD value	0.154	0.364	0.600	0.707	0.811	0.933	1.053	1.236
	0.149	0.361	0.590	0.695	0.800	0.928	1.053	1.249
Average OD value	0.152	0.363	0.595	0.701	0.806	0.931	1.053	1.243
Absoluted OD value	0	0.211	0.444	0.550	0.654	0.779	0.902	1.091



Appendix II Example Analysis

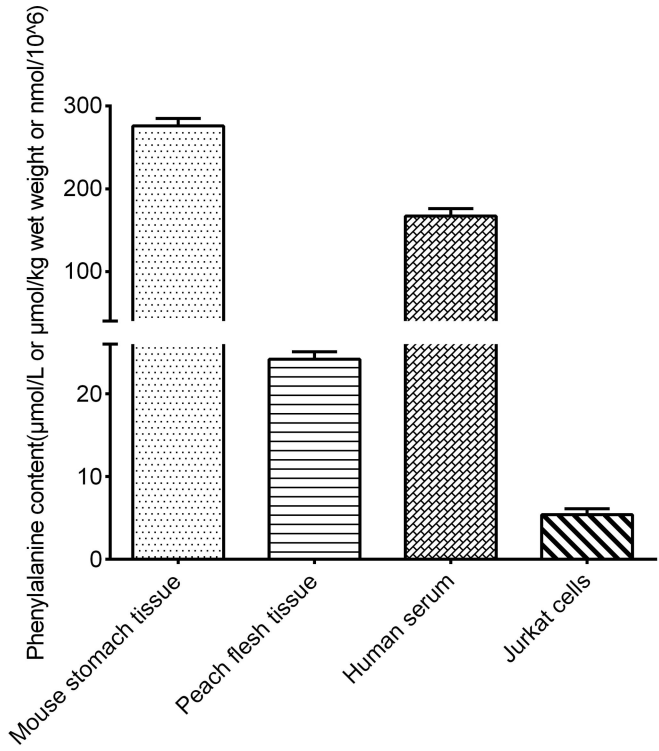
Example analysis:

Take 20 μL of human serum filtrate which dilute for 2 times and carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.0022 x - 0.0026. The OD value of the sample well is 0.879, the OD value of the control well is 0.698, and the calculation result is:

$$\text{Phe content (μmol/L)} = (0.879 - 0.698 + 0.0026) \div 0.0022 \times 2 = 166.91 \text{ μmol/L}$$

Detect 10% mouse stomach tissue homogenate (dilute for 2 times), 10% peach flesh tissue homogenate (dilute for 2 times), human serum, and 1×10^6 Jurkat cells, according to the protocol, the result is as follows:



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.