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

Catalog No：E-BC-F091

Specification：96T(40 samples)

Measuring instrument：Fluorescence Microplate Reader

(Ex/Em=535 nm/587 nm)

Detection range：0.33-200 μmol/L

Elabscience® Tryptophan (Trp) Fluorometric 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.

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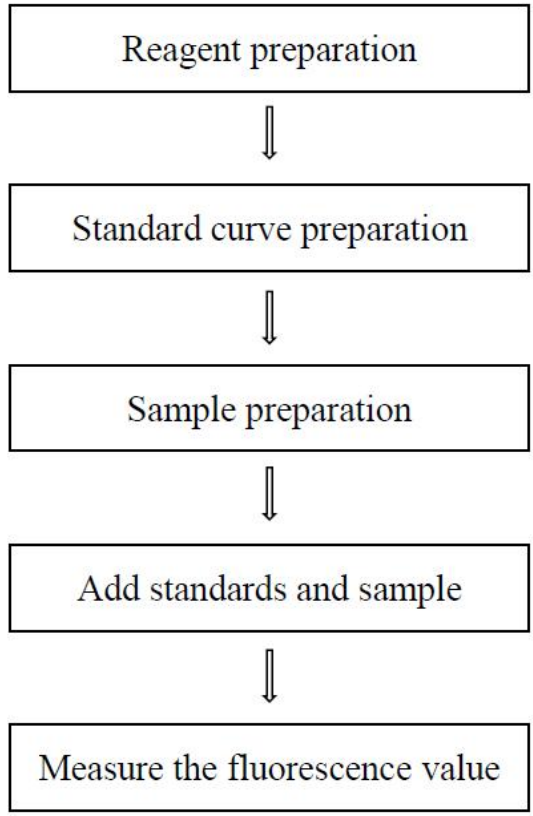
Assay summary



Intended use

This kit can be used to measure tryptophan (Trp) content in serum, plasma, tissue and cell samples.

Detection principle

Tryptophan (Trp) is an essential amino acid involved in a variety of biosynthetic processes. It is not only involved in the synthesis of a variety of proteins, but also the precursor of many bioactive substances. The enzyme catalyzes Trp in a series of reactions to produce hydrogen peroxide, which forms a red complex with the fluorescent probe. The fluorescence value of the sample at the excitation wavelength of 535 nm and the emission wavelength of 587 nm can be measured to calculate the content of Trp in the sample.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer	28 mL × 1 vial	-20°C, 12 months shading light
Reagent 2	Substrate A	Powder × 3 vials	-20°C, 12 months shading light
Reagent 3	Substrate B	Powder × 3 vials	-20°C, 12 months shading light
Reagent 4	Probe	1.2 mL × 2 vials	-20°C, 12 months shading light
Reagent 5	2 mmol/L Standard Solution	1 mL × 2 vials	-20°C, 12 months
	Black 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:

Fluorescence microplate reader (Ex/Em=535 nm/587 nm), Incubator(37℃), 3 KD ultrafiltration tube

Reagents:

Normal saline (0.9% NaCl)

Reagent preparation

① Equilibrate all the reagents to room temperature before use.

② The preparation of substrate A working solution：

Dissolve one vial of substrate A with 2.7 mL of buffer, mix well to dissolve. Store at 2-8°C for a week protected from light.

③ The preparation of substrate B working solution：

Dissolve one vial of substrate B with 4 mL of buffer, mix well to dissolve. Store at 2-8°C for a week protected from light.

④ The preparation of 200 μmol/L standard solution:

Dilute 100 μL of 2 mmol/L standard solution with 900 μL of double distilled water, mix well. Store at 2-8°C for a week.

⑤ The preparation of standard curve：

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

Dilute 200 μmol/L standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 40, 80, 100, 120, 140, 160, 200 μmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**40**	**80**	**100**	**120**	**140**	**160**	**200**
200 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

Serum, plasma sample:

① Prepare serum/plasma as the common method.

② Add sample to 3 KD ultrafiltration tube. Centrifuge at 12000×g for 15 min.

③ Take the filtered sample supernatant and preserve it on ice for detection.

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 20 mg tissue in 180 μL normal saline (0.9% NaCl) with a dounce homogenizer at 4°C.

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

⑤ Add sample to 3 KD ultrafiltration tube. Centrifuge at 12000×g for 15 min.

⑥ Take the filtered sample supernatant and preserve 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 Wash cells with normal saline (0.9% NaCl).

③ Homogenize 1×10^6 cells in 200 μL normal saline (0.9% NaCl) with a dounce homogenizer at 4°C.

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

⑤ Add sample to 3 KD ultrafiltration tube. Centrifuge at 12000×g for 15 min.

⑥ Take the filtered sample supernatant and preserve 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
Rabbit serum	1
Rat serum	1
Mouse plasma	1
Bovine serum	1
Human serum	3-8
Rat plasma	1
10% Mouse liver tissue homogenization	1
10% Rat liver tissue homogenization	1
10% Rat heart tissue homogenization	1
10% Mouse lung tissue homogenization	1
0.193 × 10^6 Hela cell	1
0.7 × 10^6 Jurkat cell	1
0.446 × 10^6 RAW cell	1
0.708 × 10^6 Molt cell	1

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 standard with different concentrations into the wells.

Sample well: add 20 μL of sample into the wells.

Control well: add 20 μL of sample into the wells.

② Add 20 μL of probe into each well.

③ Add 100 μL of substrate A working solution into standard wells and sample wells.

④ Add 100 μL of buffer into control wells.

⑤ Add 100 μL of substrate B working solution into each well.

⑥ Mix fully with microplate reader for 5 s, and incubate at 37°C for 30 min protected from light. Measure the fluorescence intensity at the excitation wavelength of 535 nm and the emission wavelength of 587 nm.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

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

3. Plot the standard curve by using absoluted fluorescence 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. Serum and plasma samples:

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

2. Tissue sample:

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

3. Cell sample:

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

[Note]

ΔF: The absolute fluorescence value of sample, $F_{sample} - F_{control}$.

m: The weight of tissue, g.

n: The number of cell sample/10^6.

V: The volume of buffer solution in the preparation step of tissue or cell, 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)	50.00	90.00	150.00
%CV	3.4	3.6	4.7

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)	50.00	90.00	150.00
%CV	5.6	4.8	2.5

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 93.6%.

	Standard 1	Standard 2	Standard 3
Expected Conc.(μmol/L)	50	90	150
Observed Conc.(μmol/L)	43.5	85.5	148.5
Recovery rate (%)	87	95	99

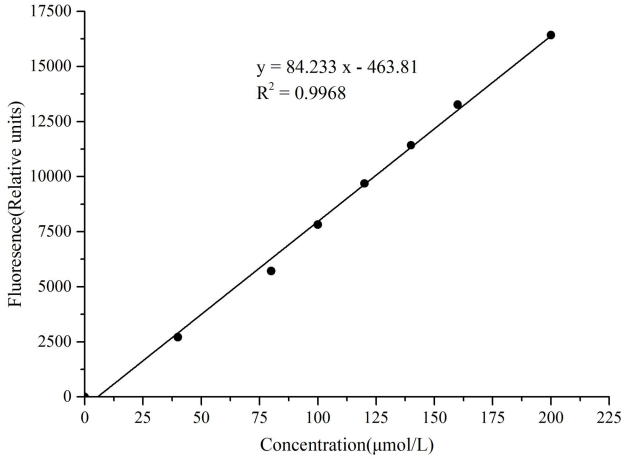
Sensitivity

The analytical sensitivity of the assay is 0.33 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 fluorescence 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	40	80	100	120	140	160	200
Fluorescence value	1914	4652	7441	9680	11456	13341	15252	18445
	1900	4585	7804	9781	11728	13316	15136	18211
Average fluorescence value	1907	4619	7622	9730	11592	13328	15174	18328
Absoluted fluorescence value	0	2712	5715	7823	9685	11421	13267	16421



Appendix II Example Analysis

Example analysis:

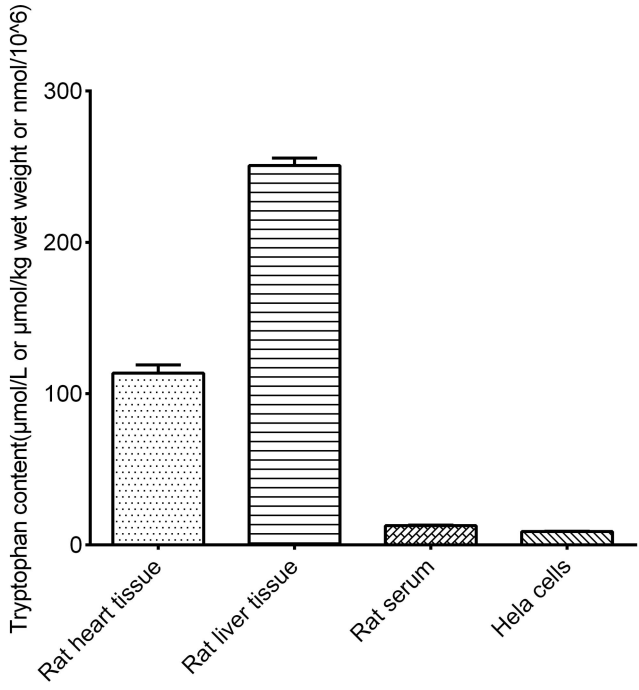
Take 20 μL of 10% rat liver tissue homogenate and carry the assay according to the operation steps. The results are as follows:

standard curve: y = 84.233x - 463.81, the average fluorescence value of the sample is 4828, the average fluorescence value of the control is 2951, and the calculation result is:

$$\text{Trp content (μmol/kg wet weight)} = (4828 - 2951 + 463.81) \div 84.233 \times 0.9 \div 0.1$$

$$= 250.11 \text{ μmol/kg wet weight}$$

Detect 10% rat heart tissue homogenate, 10% rat liver tissue homogenate, rat serum and 1×10^6 Hela 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.