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

Catalog No：E-BC-K1402-M

Specification：48T(46 samples)/96T(94 samples)/96T×5(478 samples)

Measuring instrument：Microplate reader(500-510 nm)

Detection range：0.16-15.57 U/L

Elabscience® Tyrosinase Activity 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.

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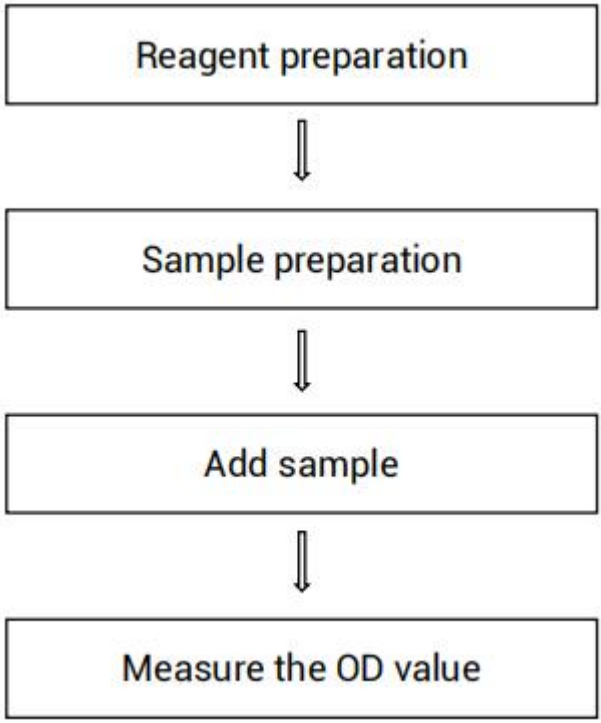
Assay summary



Intended use

The kit is suitable for detecting the activity of tyrosinase in serum, plasma, animal tissues and plant tissues.

Detection principle

Tyrosinase is widely distributed in higher animals and plants, primarily responsible for the browning of vegetables, beverages, and fruits, as well as the production of melanin in animals.

Tyrosinase catalyzes the conversion of dopamine to dopaquinone. Dopaquinone forms a pink complex with MBTH. The tyrosinase activity in the sample can be calculated by measuring the rate of increase in OD value at 505 nm.

Kit components & storage

Item	Component	Size 1 (48 T)	Size 2 (96 T)	Size 3 (96 T×5)	Storage
Reagent 1	Buffer Solution	45 mL × 2 vials	45 mL × 4 vials	45 mL × 20 vials	2-8°C,12 months
Reagent 2	Substrate	Powder × 2 vials	Powder × 4 vials	Powder × 20 vials	2-8°C, 12 months, shading light
Reagent 3	Chromogenic Agent	Powder × 2 vials	Powder × 4 vials	Powder × 20 vials	2-8°C, 12 months, shading light
	Microplate	48 wells	96 wells	96 wells×5	No requirement
	Plate Sealer	2 pieces		7 pieces	
	Sample Layout Sheet	1 piece		7 pieces	

Note: All the reagents should be stored according to the table. The reagents from different kits can not be mixed or used interchangeably. For liquid reagents with small volumes or powders, centrifuge them before use to prevent loss.

Instruments

Microplate reader (500-510 nm, optimum wavelength: 505 nm), Incubator

Materials required but not provided

DMSO, PBS（0.01 M，pH 7.4）

Reagent preparation

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

② Substrate Working Solution preparation:
Dissolve one vial of Substrate with 7.6 mL of Buffer Solution. Mix well to dissolve. The Substrate Working Solution should be freshly prepared before use. Stable for 8 h protected from light.

③ Chromogenic Working Solution preparation：
Dissolve one vial of Chromogenic Agent with 0.14 mL of DMSO. Mix well to dissolve. Then add 3.36 mL Buffer Solution and mix well. The Chromogenic Working Solution should be freshly prepared before use. Stable for 8 h protected from light.

④ Working Solution preparation:
Before testing, please prepare sufficient Working Solution according to the test wells. For example, prepare 1250 μL of Working Solution (mix well 1000 μL of Substrate Working Solution and 250 μL of Chromogenic Working Solution). The Working Solution should be freshly prepared before use. Stable for 8 h protected from light.

Sample preparation

Serum and plasma: detect directly.

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 Buffer Solution with a dounce homogenizer at 4°C.

④ Centrifuge at 10000×g for 10 min at 4°C. Collect supernatant and keep it on ice for detection and detect within 8 h.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

Dilution of sample

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

Sample type	Dilution factor
10% Mouse kidney tissue homogenization	1
10% Mouse liver tissue homogenization	1
10% Potato tissue homogenization	5-20
10% Sweet potato tissue homogenization	2-10
Human serum	1

Note: The diluent is Buffer Solution. For the dilution of other sample types, please do pretest to confirm the dilution factor.

Operating steps

① Control well: Add 20 μL of Buffer Solution to the corresponding wells.
Sample well: Add 20 μL of sample to the corresponding wells.

② Add 250 μL of Working Solution to each well.

③ Shake the microplate for 5 seconds to ensure complete mixing. Measure the OD value of each well at 505 nm, as A_1. Incubate at 37°C for 20 min and measure the OD value of each well at 505 nm, as A_2.

Calculation

The sample:

1. Serum and plasma samples:

$$\text{Tyrosinase activity (U/L)} = (\Delta A_{sample} - \Delta A_{control}) \times V_1 \div \varepsilon \div d \div V_2 \div T \times 10\text{^}6 \times f$$

$$= (\Delta A_{sample} - \Delta A_{control}) \times 31.14 \times f$$

2. Tissue sample:

$$\text{Tyrosinase activity (U/gprot)} = (\Delta A_{sample} - \Delta A_{control}) \times V_1 \div \varepsilon \div d \div V_2 \div T \div$$

$$= (\Delta A_{sample} - \Delta A_{control}) \times 31.14 \div C_{pr} \times f$$

[Note]

ΔA_{sample}: The OD value of the sample well changes., $A_2 - A_1$.

$\Delta A_{control}$: The OD value of the control well changes., $A_2 - A_1$.

ε: The molar extinction coefficient, 2.89×10^4 L/mol/cm.

d: Optical path of microplate wells, 0.75 cm.

V_1: The volume of reaction system, 270 μL.

V_2: The volume of sample, 20 μL.

T: Reaction time, 20min.

C_{pr}: The concentration of protein in sample, gprot/L.

10^6: 1 mol = 1×10^6 μmol.

f: Dilution factor of sample before tested.

31.14: Simplified value.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three potato 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 (U/L)	2	8	13
%CV	0.7	0.4	0.6

Inter-assay Precision

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

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	2	8	13
%CV	6.4	8.1	6.8

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 100%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (U/L)	2	8	13
Observed Conc. (U/L)	2	7.84	13.26
Recovery rate (%)	100	98	102

Sensitivity

The analytical sensitivity of the assay is 0.16 U/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.

Appendix Π Example Analysis

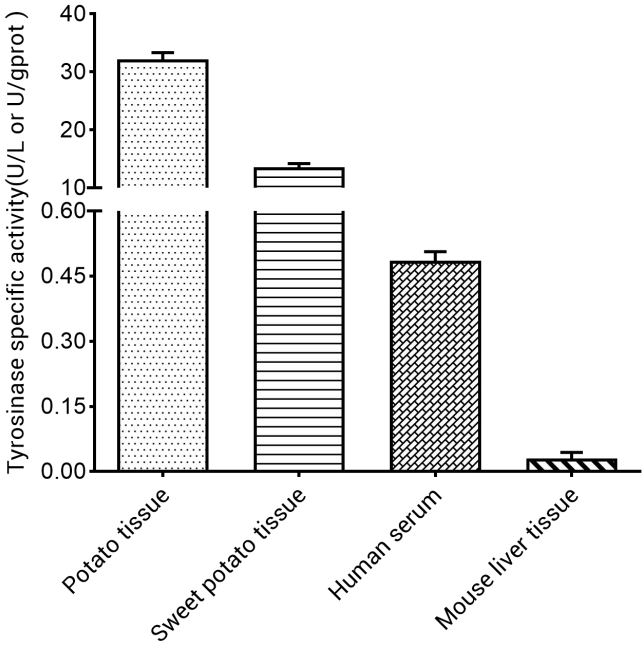
Example analysis：

Take 20 μL of the 10-fold diluted 10% potato tissue homogenate to the well of microplate. Proceed according to the operating steps. The results are as follows:

The A_1 value of the sample is 0.066, the A_2 value of the sample is 0.313, ΔA_{sample} = 0.313 – 0.066 = 0.247; the A_1 value of the control is 0.042, the A_2 value of the control is 0.048, $\Delta A_{control}$ = 0.048 - 0.042 = 0.006, the concentration of protein is 2.4 gprot/L, and the calculation result is:

Tyrosinase activity (U/gprot) = (0.247 - 0.006) × 31.14 ÷ 2.4 × 10= 31.27 U/gprot

Detect 10% potato tissue homogenate supernatant (the concentration of protein is 2.4 gprot/L, dilute for 10 times), 10% sweet potato tissue homogenate supernatant (the concentration of protein is 1.38 gprot/L, dilute for 4 times), human serum, according to the protocol and 10% mouse liver tissue homogenate supernatant (the concentration of protein is 13.02 gprot/L), the result is as follows:


Tyrosinase specific activity(U/L or U/gprot)
40
30
20
10
0.60
0.45
0.30
0.15
0.00
Potato tissue
Sweet potato tissue
Human serum
Mouse liver tissue

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.