Mushroom Tyrosinase Inhibitor Screening Kit (Diphenolase activity)

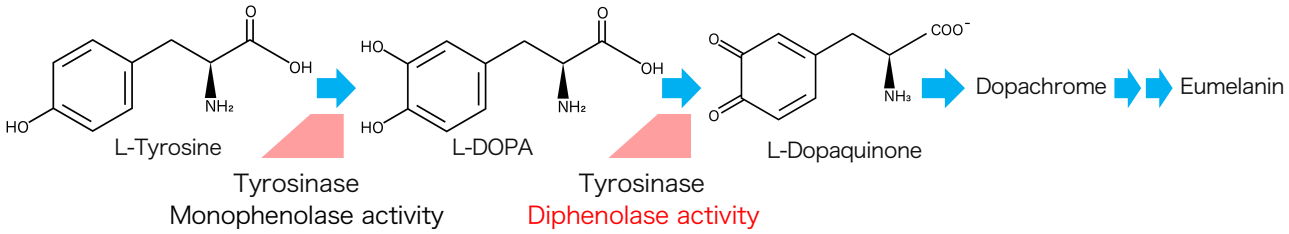
Cat. No. SL-7011 (96 tests)

1. Introduction & Applications

1-1. Introduction

Tyrosinase (EC1.14.18.1) is one of important enzyme in melanin biosynthesis and present universally in all living organisms. The enzyme catalyzes two types of reaction: the ortho-hydroxylation of monophenols, rendering into o-diphenols (monophenolase activity), and the oxidation of o-diphenols to o-quinones (diphenolase activity). Melanins are the pigments responsible for pigmentation of skin, hair, eye-lens and some regions in brain. They play role in coloration, absorption of UV radiations and electron transfer properties and prevents sun induced skin injuries. However, excessive melanogenesis results in browning in skin pigmentation and during fruit harvesting and handling. Because inhibitions of tyrosinase bring skin-whitening and plant-bleaching, tyrosinase inhibitors have been developed in the skin health research, cosmetics and agricultural industries.

Mushroom Tyrosinase Inhibitor Screening Kit (Diphenolase activity)(SL-7011) provides a rapid, simple, sensitive, and reliable test suitable for high-throughput screening of tyrosinase inhibitors. Mushroom tyrosinase catalyzes the oxidation of the DOPA as a substrate, producing a chromophore that can be detected at OD = 495 nm. In the presence of kojic Acid (Inhibitor control), a reversible inhibitor of tyrosinase, the rate of oxidation of the substrate is decreased.



1-2. Applications

Screening and characterizing tyrosinase inhibitors

2. Kit Components

Assay buffer	25 mL
Enzyme (Mushroom tyrosinase)	150 μL X 4
Substrate(L-DOPA)	600 μL
Inhibitor control (10mM Kojic acid)	300 μL
96 well microplate	1 plate

3. User Supplied Reagents and Equipment

- Microplate reader (480-510nm filter)
- Incubator (25-30˚C)
- Multichannel pipette(recommendation)

4. Storage

This kit store at -20ºC in the dark immediately upon receipt. Unused components are stable for 6 months. After use, individual prepared components stored at the following temperature. Reconstituted components are stable for 3 months.

Assay buffer	Store at 4˚C
Enzyme	Store at -20˚C
Substrate (L-DOPA)	Store at -20˚C
Inhibitor control (Kojic acid)	Store at -20˚C
96 well microplate	Store at room temperature

5. Reagent Preparation

5-1. Tyrosinase Assay Buffer
Ready to use as supplied. Equilibrate to room temperature before use.

5-2. Enzyme solution
Thaw Enzyme on ice before use. For each well, prepare 20μL Enzyme solution. Keep on ice while in use.

Assay Buffer	15μL
Enzyme	5μL

5-3. Substrate solution (The solution is prepared immediately before use)
Thaw Substrate buffer, Substrate and Enhancer at room temperature before use. For each well, prepare 60μL Substrate solution.

Substrate	5μL
Assay Buffer	55μL

5-4. Sample & Inhibitor control
Sample can be diluted to the desired test concentration with Assay buffer before use. Inhibitor control (10mM Kojic acid) is ready to use as supplied. Thaw Inhibitor control at room temperature before use. The final concentration is 2mM.

6. Experimental Protocol

Step1(Sample preparation)
Add 20μL of Control (Assay buffer), Inhibitor control and Samples for each well on 96 well microplate.

Step2(Enzyme preparation)
Add 20μL of Enzyme solution for each well and gently shake. Incubate at 25-30˚C for 10min.

Step3(Substrate preparation)
Add 60μL of Substrate solution for each well and gently shake. Incubate at 25-30˚C for 30-60 min.

Step4(Measurement)
Measure the absorbance after 30min (T30) and 60 min (T60) of incubation at 480-510nm (recommended wavelength is 495nm). The absorbance (AbsT30 & T60) of two time points is obtained.

7. Calculations

Calculate % Relative Inhibition as follows:

$$\Delta(\text{Control}) = \text{AbsT60(Control)} - \text{AbsT30(Control)}$$

$$\Delta(\text{Sample}) = \text{AbsT60(Sample)} - \text{AbsT30(Sample)}$$

$$\%\ \text{relative inhibition} = \frac{\Delta(\text{Control}) - \Delta(\text{Sample})}{\Delta(\text{Control})} \times 100$$

8. Sample of Results

Assay was performed following kit protocol.

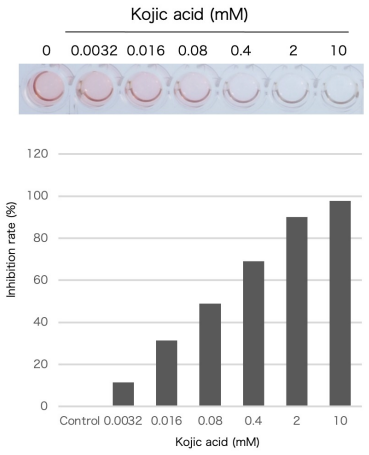


Figure1. Inhibitions of Tyrosinase activity (Diphenolase activity) with Kojic Acid

Table 1
IC50 values of the typical inhibitor components are indicated in Table 2.

Activity	Monophenolase activity	Diphenolase activity
Substrate	L-Tyrosine	L-DOPA
Kojic acid	80μM	80μM
α-Arbutin	1,700μM	No inhibition
β-Arbutin	2,000μM	No inhibition
Hydroquinone	160μM	No inhibition
Ascorbic acid	20μM	390μM

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