Tribioscience

Tyrosinase Activity Colorimetric Assay (TBS2072; 100 assays; Store at -20°C)

DESCRIPTION

Tyrosinase is a copper-binding enzyme that is expressed across a vast range of species ranging from bacteria and fungi to mammals. It is involved in two sequential reactions of the melanin synthesis pathway: first being the hydroxylation of a monophenol and second the conversion of an ortho-diphenol to a quinone. Quinone then undergoes a series of reactions including polymerization to form melanin. Tyrosinase is of great interest to the agriculture industry since it causes browning of fruits, vegetable and mushrooms, as well as to the cosmetic industry as it causes skin darkening. Development and screening of tyrosinase inhibitors is very useful for conditions such as hyperpigmentation and melasma. Tyrosinase activity is significantly increased in melanoma. Therefore, the detection of tyrosinase activity could be promising as a specific diagnostic test for melanoma and may be useful in monitoring patient response to melanoma treatments.

Tribioscience's Tyrosinase Activity Assay Kit provides a simple, sensitive assay for the measurement of tyrosinase activity in biological samples. In this assay, tyrosinase catalyzes the conversion of a phenolic substrate to a Quinone intermediate, which reacts with a probe forming a highly stable chromophore with absorbance at 510 nm. The assay can detect as low as 30 µU Tyrosinase in biological samples.

ASSAY PRINCIPLE

Substrate —(Tyrosinase)→ **Intermediate** —(Probe)→ **Chromophore (OD 510 nm)**

APPLICATION

•Measurement of Tyrosinase activity in various biological samples.

• Analysis of Tyrosinase in pathological conditions.

KIT CONTENTS

Component	100x Rxns
Tyrosinase substrate	2.5 mL
Tyrosinase Standard (400U/mL)	250 µL
Tyrosinase Probe	0.6 mL
Assay buffer	10 ml

Storage conditions: Store the kit at –20°C protected from light.
Shelf life: 12 months.

ASSAY PROCEDURES

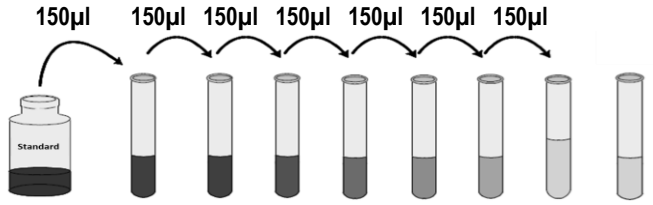
1. Sample Preparation

Collect cell culture supernatant, serum, plasma, urine and other biological fluids. Deproteinizing the samples using 10 kDa Spin Column (Amicon Ultra 0.5 mL, Cat. No. UFC501024). Briefly, add sample to the spin column, centrifuge at 10,000 x g for 5 min. at 4°C. Collect the filtrate. Add 50 µl of filtrate into desired well(s) in 96-well plate.

2. Standard Preparation as Fig.1:

2.1 Label 8x 1.5mL tubes 1-8 for a standard curve as shown in the diagram below.
2.2 Add 150 µL of Assay Buffer into to Std 1-8.
2.3 Add 150 µL of tyrosinase Standard Stock (400U/mL) solution to Std1, then carry out a 2x serial dilution for Std 2-7. Leave Std 8 as the 0 standard (the assay buffer alone). The standards concentrations are 200, 100, 50, 25, 12.5, 6.25, 3.125 and 0 µM for Std 1-8.

Fig.1 Diagram for Tyrosine Standard Preparation



	Std1	Std2	Std3	Std4	Std5	Std6	Std7	Std8
Assay Buffer (µL)	150	150	150	150	150	150	150	150
Addition	Stock	Std1	Std2	Std3	Std4	Std5	Std6	
Addition Vol.(µL)	150	150	150	150	150	150	150	
Final Conc (U/mL)	200	100	50	25	12.5	6.25	3.125	0

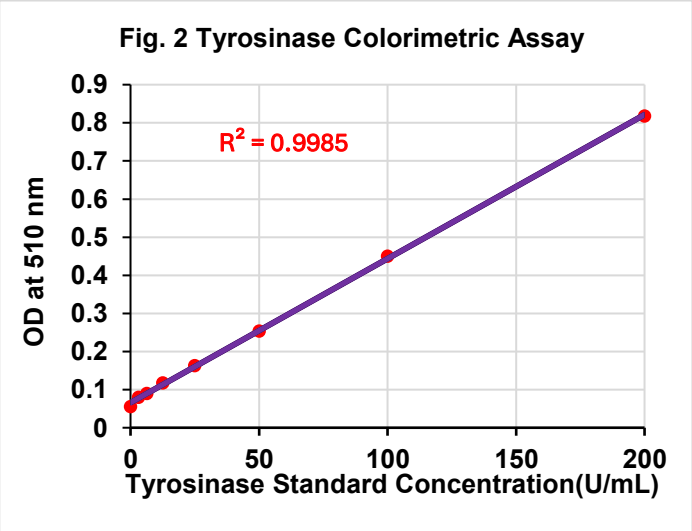
2.4 Load the samples: Pipet 50µL of standards, sample or controls into indicated well of the plate in duplicate manner *(Note: recommend running a pilot study to determine the optimal concentration of sample within the assay standard curve range)*.
2.5 Prepare Enzyme working solution for each well by mixing the reagent as following:
• 25 µL Assay Buffer
• 20 µL Substrate
• 5 µL Probe
Mix, add each well.
For 96well plate assay: mix 2.5 mL Assay Buffer + 200uL substrate and 500uL probe, mix.
2.6 Begin the reactions. Add 50µL of above working solution to each microplate well containing the standards, controls, and samples.
2.7 Incubate the reactions. Incubate at 37°C for 60 - 90minutes protected from light.
2.8 Measure absorbance using a microplate reader at 510 nm.
2.9 Correct for background absorbance. For each point, subtract the value derived from the 0 control.

Calculation

Subtract the blank value (0µM Standard) from the standard values and plot the ΔOD against standard concentrations. Determine the slope and calculate the Tyrosinase activity of the Sample using the equation obtained from the linear regression of the standard curve.

Tyrosinase (U/mL) = N x (Rsample- Rblank)/Slope

Where: Rsample and Rblank are optical density readings of the sample and blank, respectively. N is the sample dilution factor.



RELATED PRODUCTS:

Tyrosine Colorimetric Assay (TBS2070)
ATP Activity Assay (TBS2010)
Hydrogen Peroxidase Activity Assay Kit (TBS2067)
HRP Fluorescence -System（TBS5026）
Tryptase Activity Assay (TBS2101)
β-Hexosaminidase Activity Assay (TBS2105)
Cytochrome C Oxidase Activity Assay (TBS2115)
Fast Glucose Determination Colorimetric/Fluorometric Assay (TBS2087)
Glucose Oxidase Activity Colorimetric/Fluorometric Assay (TBS2088)
Non-esterified Fatty Acid Assy (TBS2203)
Glycerol Colorimetric / Fluorometric Assy (TBS2204)
Protein Assay Kits (TBS2005)
Cell Nuclear Extract kit (TBS6025)

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