Tyrosinase Activity Colorimetric Microplate Assay Kit

Cat #: orb1881042 (manual)

Detection and Quantification of Tyrosinase Activity in Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

For research use only. Not for diagnostic or therapeutic procedures.

INTRODUCTION

Tyrosinase (EC 1.14.18.1) is a copper-binding enzyme expressed across a wide range of species, from bacteria and fungi to mammals. It catalyzes two sequential reactions in the melanin synthesis pathway: first, the hydroxylation of a monophenol, and second, the oxidation of an ortho-diphenol to a quinone.

The quinone then undergoes a series of reactions, including polymerization, ultimately forming melanin. Tyrosinase is of significant interest in the agricultural industry because it is responsible for the browning of fruits, vegetables, and mushrooms. It is also important in the cosmetic industry due to its role in skin darkening. Consequently, the development and screening of tyrosinase inhibitors are valuable for treating conditions such as hyperpigmentation and melasma.

Tyrosinase activity is significantly elevated in melanoma. Therefore, detecting tyrosinase activity may serve as a promising diagnostic approach for melanoma and could be useful for monitoring patient response to melanoma treatments.

The Tyrosinase Activity Colorimetric Microplate Assay Kit provides a sensitive method for measuring tyrosinase activity in various sample types. In this assay, tyrosinase catalyzes the conversion of a phenolic substrate into a quinone intermediate, a stable chromophore that exhibits absorbance at 492 nm.

KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer	30 ml x 1	4 °C
Substrate	Powder x 1	4 °C
Positive Control	Powder x 1	-20 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

Note:

Substrate: add 10 ml distilled water before use, vortex to dissolve, store at 4°C for 1 month after reconstitution.

Biorbyt Ltd
7 Signet Court, Swann Road, Cambridge, CB5 8LA. United Kingdom
Email: info@biorbyt.com | Phone: +44 (0)1223 859-353 | Fax: +44 (0)1223 280-240

Biorbyt LLC
68 TW Alexander Drive, Durham, NC 27713. United States
Email: info@biorbyt.com | Phone: +1 (415) 906-5211 | Fax: +1 (415) 651-8558

Positive Control: add 0.2 ml Assay Buffer to dissolve before use, store at -80°C for 1 month after reconstitution.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 492 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Ice
7. Centrifuge
8. Timer

SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay buffer for 5×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times); centrifuged at 8000g 4°C for 10minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For tissue samples

Weigh out 0.1g tissue, homogenize with 1mlAssay buffer on ice, centrifuged at 8000g 4°C for 10minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For serum, plasma samples or plant juice

Add 0.1 ml serum, plasma or plant juice into 0.9 ml Assay buffer on ice, centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

ASSAY PROCEDURE

Warm Reaction Buffer and Substrate to 37°C before use. Add following reagents into the microplate:

Reagent	Sample	Control	Positive Control
Sample	50 µl	--	--
Sample (boiled)	--	50 µl	--
Positive Control	--	--	50 µl
Reaction Buffer	150µl	150µl	150µl
Substrate	50µl	50µl	50µl
Mix, put it in the oven, 37°C for 3minutes, record absorbance measured at 492 nm.			

Note:

1) For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more sample into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

2) Reagents must be added step by step, cannot be mixed and added together.

CALCULATION

Unit Definition: one unit is defined as the OD value changed 0.001per minute in the reaction system.

1. According to the protein concentration of sample

$Tyrosinase(U/mg) = (OD_{Sample} - OD_{Control}) \times V_{Total} / (C_{Protein} \times V_{Sample}) / 0.001 / T$

$= 1333.3 \times (OD_{Sample} - OD_{Control}) / C_{Protein}$

2. According to the weight of sample

$Tyrosinase\ (U/g) = (OD_{Sample} - OD_{Control}) \times V_{Total} / (W \times V_{Sample} / V_{Assay}) / 0.001 / T$

$= 1333.3 \times (OD_{Sample} - OD_{Control}) / W$

3. According to the quantity of cell or bacteria

$Tyrosinase\ (U/10^4) = (OD_{Sample} - OD_{Control}) \times V_{Total} / (N \times V_{Sample} / V_{Assay}) / 0.001 / T$

$= 1333.3 \times (OD_{Sample} - OD_{Control}) / N$

4. According to the volume of serum, plasma or plant juice

$Tyrosinase(U/ml) = (OD_{Sample} - OD_{Control}) \times V_{Total} / (V \times V_{Sample} / V_{Assay}) / 0.001 / T$

$= 1333.3 \times (OD_{Sample} - OD_{Control}) / V$

$C_{Protein}$: the protein concentration, mg/ml

W: the weight of sample, g

V: the volume of sample, ml

N: the quantity of cell or bacteria, $N \times 10^4$

V_{Total}: the volume of sample, 0.2 ml

V_{Sample}: the volume of sample, 0.05 ml

V_{Assay}: the volume of Assay buffer, 1 ml.

T: the reaction time, 3 minutes.

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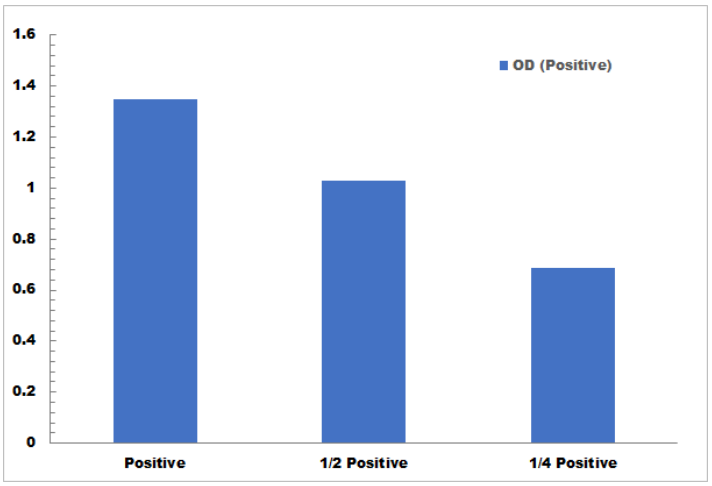
TYPICAL DATA



Positive Control reaction in 96-well plate assay with decreasing the concentration

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