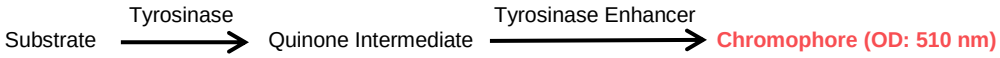
Tyrosinase Activity Assay Kit (Colorimetric)

rev 04/21

(Catalog # K742-100; 100 assays; Store at – 20 ºC)

I. Introduction:

Tyrosinase (EC 1.14.18.1) 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, therefore 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. **BioVision's Tyrosinase Activity Assay Kit** is a simple one-step, plate-based 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 the tyrosine enhancer forming a highly stable chromophore with absorbance at 510 nm. The assay can detect as low as 30 µU Tyrosinase in biological samples.



II. Application:

- Measurement of tyrosinase activity in cell and tissue lysates using a 96-well plate format.

III. Sample Type:

- Cell lysate (e.g. Melanoma cells)
- Plant tissue lysate (e.g. potato)
- Recombinant enzyme
- Purified protein

IV. Kit Contents:

Components	K742-100	Cap Code	Part Number
Tyrosinase Assay Buffer	25 ml	WM	K742-100-1
Tyrosinase Substrate	1.1 ml	Orange	K742-100-2
Tyrosinase Enhancer	1 vial	Green	K742-100-3
Chromophore Standard (1 mM)	400 µl	Yellow	K742-100-4
Tyrosinase Positive Control	1 vial	Blue	K742-100-5

V. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer
- Distilled water

VI. Storage Conditions and Reagent Preparation:

Upon arrival, store the kit at -20 °C, protected from light. Briefly centrifuge small vials before opening. Read entire protocol before performing the assay.

- **Tyrosinase Assay Buffer:** Warm to room temperature (RT) before use.
- **Tyrosinase Substrate:** Thaw on ice before use. Divide into aliquots and store at -20 ºC, proteceted from light. *Do not expose to light.*
- **Tyrosine Enhancer**: Reconstitute the vial in 550 µl water. Divide into aliquots and store at -20 ºC.
- **Chromophore Standard (1 mM):** Ready to use as supplied.
- **Tyrosinase Positive Control:** Reconstitute the vial in 220 µl Tyrosinase Assay Buffer. Divide into aliquots and store at -20 °C. Avoid repeated freeze/ thaw cycles. Use within two months. Keep on ice while in use

VII. Tyrosinase Activity Assay Protocol:

1. **Sample Preparation**: Homogenize cells (8 x 10^6 cells) or tissue (50 mg) with 500 µl ice-cold Tyrosinase Assay buffer to perform lysis and keep on ice for 10 minutes followed by centrifugation at 10,000 x g for 15 minutes at 4 ºC. Collect the supernatant (lysate) and estimate protein concentration using preferred method. *We recommend BCA protein assay kit (BV# K813-2500). Protein concentration should range between 1 and 2.5 µg / µl*). Dilute the lysate if needed using Tyrosinase Assay Buffer. Use the samples for activity analysis immediately; if that is not possible, they may be stored at -80 ºC. Prepare two wells for each sample labeled "**Sample Background Control**" (SBC) and "**Sample**" (S). Add the same volume (2 - 25 µl, i.e. 5 – 25 µg protein) into each of these wells. **For Positive Control,** add 2 µl of the provided Tyrosinase Positive Control into the desired well. Adjust volume in each well to 50 µl with Tyrosinase Assay Buffer. **For Assay Background Control (i.e., substrate background)**, add 50 µl of Tyrosinase Assay Buffer to a well. **Note:** For unknown samples, we suggest testing several concentrations to ensure the readings are within the Standard Curve range.
2. **Chromophore Standard Curve Generation:** Add 0, 2, 4, 6, 8, 10 µl of the 1 mM Chromophore Standard into a series of wells in a clear 96-well plate to obtain 0, 2, 4, 6, 8 and 10 nmol/well. Adjust the volume of each well to 100 µl with Tyrosinase Assay Buffer. **Optional (for samples that have low activity):** Dilute 10 µl of the Chromophore Standard with 90 µl of Tyrosinase Assay Buffer to

obtain 100 µM Chromophore Standard. Then add 0, 2, 4, 6, 8, 10 µl of the diluted Chromophore Standard into a series of wells in a clear 96-well plate to obtain 0, 200, 400, 600, 800 and 1000 pmol/well of Chromophore. Adjust the volume of each well to 100 µl with Tyrosinase Assay Buffer.

3. **Reaction Mix Preparation:** Mix enough reagents for the number of assays to be performed. Add "SBC Mix" to the "Sample Background Control" wells and "Reaction Mix" to Assay Background Control (substrate background), Sample, and Positive Control wells. For each well, prepare 50 µl:

	SBC Mix	Reaction Mix
Tyrosinase Assay Buffer	45 µl	35 µl
Tyrosinase Substrate	-	10 µl
Tyrosinase Enhancer	5 µl	5 µl

Mix well. Add the reaction mix to wells of the 96-well clear plate. **Do not add reaction mix to the Standard Curve wells.**

Notes:

a. Have the plate reader ready at 37 °C, at Abs 510 nm on kinetic mode set to record absorbance every 30 seconds.
b. Prepare reaction mix immediately before adding it to wells.

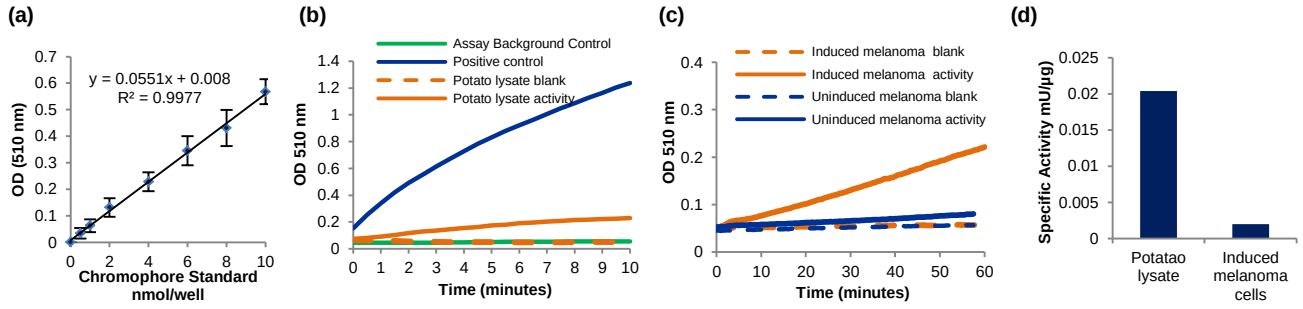
4. **Measurement:** Immediately start recording absorbance at 30 second intervals for 10-15 minutes for samples with high tyrosinase activity and for 60-90 minutes for samples with low tyrosinase activity. Standard Curve may be read in end point mode.

5. **Calculation:** Subtract Sample Background Control OD values form sample OD values. If the Assay Background Control OD values are higher than Sample Background Control, subtract those values from sample OD values instead. Estimate the amount of chromophore formed using the Standard Curve. Calculate ΔM, which is the change in amount of chromophore between time t_1 and t_2, such that t_1 and t_2 both fall in the linear portion of the reaction. Tyrosinase activity may be calculated using the following equation:

Sample Tyrosinase specific activity = ΔM / (Δt x P) (nmol / (min x µg)) = mUnits / µg = Units / mg

Where: **ΔM =** linear change in amount of chromophore during Δt (nmol)
Δt = $t_2 - t_1$ (min)
P = sample protein content added to well (µg)

Unit Definition: One unit of Tyrosinase is the amount of enzyme that produces1 µmol of chromophore per minute at pH 7.4 at 37 °C.



Figures 1: (**a**) Chromophore Standard Curve (**b**) Enzyme kinetics for tyrosinase positive control and for potato lysate (16 µg protein). (**c**) Enzyme kinetics for tyrosinase activity in uninduced melanoma cells cultured in EMEM + 10% FBS (15 µg protein), and melanoma cells induced for increased tyrosinase activity by culturing for 4 days in EMEM + 0.5% FBS, supplemented with 500 µM cAMP, 100 µM PDE inhibitor IBMX and 100 µM Cu^{2+} (10 µg protein). **(d)** Tyrosinase specific activity in potato lysate and melanoma cells.

VIII. Related Products:

Tyrosine Colorimetric Assay Kit (K573)
Myeloperoxidase (MPO) Colorimetric Activity Assay Kit (K744)
Tyrosinase Inhibitor Screening Kit (Colorimetric) (K575)
Protein Tyrosine Phosphatase Activity Kit (Fluorometric) (K829)

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