Technical Bulletin

Tyrosinase Activity Assay Kit (Colorimetric)

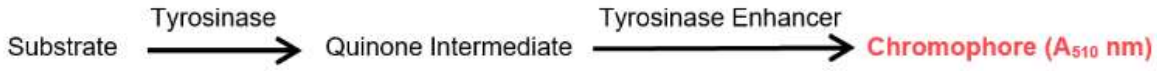
Catalog Number MAK395

Product 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. The first reaction is the hydroxylation of a monophenol, and the second reaction is the conversion of an ortho-diphenol to a quinone. Quinone subsequently 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 tyrosinase 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.

The Tyrosinase Activity Assay Kit is a simple one-step, plate-based assay for the measurement of tyrosinase activity. 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.

The kit is suitable for the measurement of tyrosinase activity in cell lysates (e.g., melanoma cells), tissue lysates, plant tissue lysates (e.g. potato), recombinant enzymes and purified proteins.



Components

The kit is sufficient for 100 colorimetric assays in 96-well plates.

- Tyrosinase Assay Buffer 25 mL
  Catalog Number MAK395A
- Tyrosinase Substrate 1.1 mL
  Catalog Number MAK395B
- Tyrosinase Enhancer 1 vial
  Catalog Number MAK395C
- Chromophore Standard (1 mM) 400 μL
  Catalog Number MAK395D
- Tyrosinase Positive Control 1 vial
  Catalog Number MAK395E

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (including multichannel pipettor)
- 96-well clear flat-bottom plate. Cell culture or tissue culture treated plates are **not** recommended.
- Spectrophotometric multiwell plate reader
- Dounce tissue grinder set (Catalog Number D9063 or equivalent)
- Refrigerated microcentrifuge capable of RCF ≥10,000 × *g*
- Bicinchoninic Acid Kit for Protein Determination (Catalog Number BCA1)

Precautions and Disclaimer

For Research Use Only. Not for uses in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store kit at -20 ºC, protected from light.

Preparation Instructions

Briefly centrifuge small vials prior to opening.

Tyrosinase Assay Buffer: Warm to room temperature prior to use. Chill an appropriate amount of Tyrosinase Assay Buffer for use in Sample Preparation.

Tyrosinase Substrate: Thaw on ice prior to use. Aliquot and store the remaining stock at -20 ºC in dark vials. **Do not expose to light.**

Tyrosine Enhancer: Reconstitute in 550 µL of purified water. Aliquot and store the remaining stock at -20 ºC.

Chromophore Standard (1 mM): Ready to use as supplied.

Tyrosinase Positive Control: Dissolve the lyophilized tyrosinase in 220 µL of Tyrosinase Assay Buffer. Aliquot and store at -20 ºC. Avoid repeated freeze/ thaw cycles. Use within two months of reconstitution. Keep on ice while in use.

Procedure

Sample Preparation

1. Homogenize cells (8×10^6 cells) or tissue (50 mg) with 500 µL of ice-cold Tyrosinase Assay Buffer to perform lysis and keep on ice for 10 minutes.
2. Centrifuge at 10,000 × *g* for 15 minutes at 4 ºC.
3. Collect the supernatant (lysate) and determine the protein concentration using preferred method (BCA is recommended). Protein concentration should range between 1-2.5 µg/µL.
4. Dilute the lysate if needed using Tyrosinase Assay Buffer. Use the samples for activity analysis immediately. If that is not possible, samples may be stored at -80 ºC.
5. Prepare two wells for each sample labeled "Sample Background Control" (SBC) and "Sample" (S). Add the same volume (2-25 µL, 5–25 µg protein) into each of these wells. Adjust total volume in each well to 50 µL with Tyrosinase Assay Buffer, mix well. For unknown samples, test several concentrations to ensure the readings are within the Standard Curve range.

Positive Control

Add 2 µL of Tyrosinase Positive Control plus 48 µL of Tyrosinase Assay Buffer into the desired well. Mix well.

Assay Background Control (Substrate Background)

Add 50 µL of Tyrosinase Assay Buffer to desired well.

Standard Curve Preparation (0-10 nmol/ well)

Prepare Chromophore Standards using 1 mM Chromophore Standard stock according to Table 1. Mix well.

Table 1.
Preparation of Chromophore Standards

Well	1 mM Stock	Tyrosinase Assay Buffer	Chromophore (nmol/well)
1	0 µL	100 µL	0
2	2 µL	98 µL	2
3	4 µL	96 µL	4
4	6 µL	94 µL	6
5	8 µL	92 µL	8
6	10 µL	90 µL	10

Optional Standard Curve for Low-Activity Samples (0-1000 pmol/well)

Dilute the 1 mM Chromophore Standard by adding 10 µL to 90 µL of Tyrosinase Assay Buffer to obtain a 100 µM Chromophore Standard. Prepare standard wells according to Table 2.

Table 2.
Preparation of Chromophore Standards for low activity samples

Well	100 µM Stock	Tyrosinase Assay Buffer	Chromophore (pmol/well)
1	0 µL	100 µL	0
2	2 µL	98 µL	200
3	4 µL	96 µL	400
4	6 µL	94 µL	600
5	8 µL	92 µL	800
	10 µL	90 µL	1000

Reaction Mix

1. Prior to preparing reaction mixes, set the plate reader at 37 °C, 510 nm on kinetic mode set to record absorbance every 30 seconds.
2. Prepare reaction mix immediately before adding it to wells. Mix enough reagents for the number of assays to be performed.
   a. For each well containing Sample Background Control, prepare 50 µL of SBC Mix according to Table 3. Mix well.
   b. For each well containing Sample, Assay Background Control, or Positive Control, prepare 50 µL of Reaction Mix according to Table 3. Mix well.

Table 3.
Reaction Mix Preparation

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

3. Add 50 µL of the Reaction Mix to each well of the 96-well clear plate containing Sample, Assay Background Control, or Positive Control. Add 50 µL of the SBC Mix to each well containing Sample Background Control. Mix well. Do not add Reaction Mix to Standard wells.

Measurement

Immediately start recording absorbance at 510 nm (A_{510}) 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.

Results

1. Subtract Sample Background Control A_{510} values from Sample A_{510} values. If Assay Background Control A_{510} values are higher than Sample Background Control, subtract those values from Sample A_{510} values instead.
2. Estimate the amount of chromophore formed using the standard curve. Calculate ΔM, which is the change in amount of chromophore between time T1 and T2, such that T1 and T2 both fall in the linear portion of the reaction.
3. Tyrosinase activity may be calculated using the following equation:

   Tyrosinase specific activity (nmol/(min × μg) or mUnits/μg or Units/mg) =

   $$\Delta M / (\Delta T \times P)$$

where:

ΔM = Linear change in amount of chromophore during ΔT (nmol)

ΔT = T2 – T1 (minutes)

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.

Figure 1.
Typical Chromophore Standard Curve

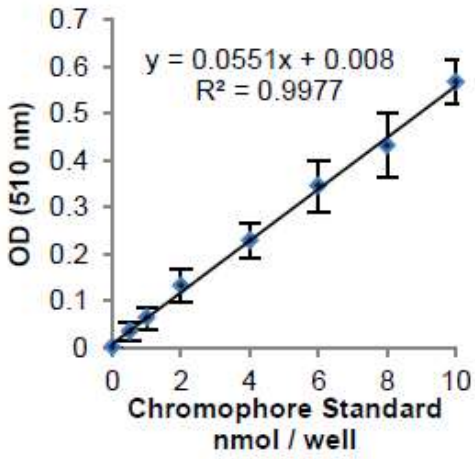


Figure 2.
Enzyme kinetics for tyrosinase positive control and for potato lysate (16 μg protein).

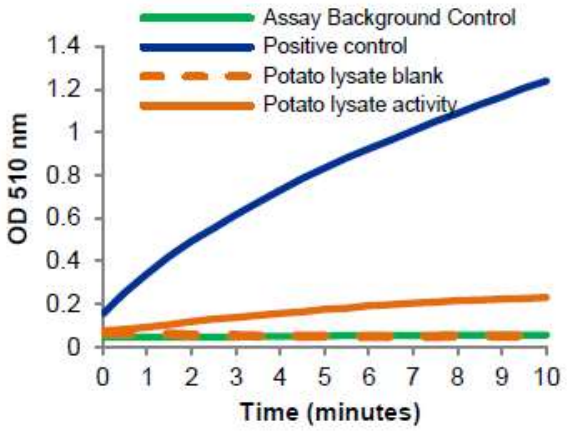


Figure 3.
Enzyme kinetics for tyrosinase activity in uninduced melanoma cells cultured in EMEM + 10% FBS (15 μg protein) and melanoma cells induced for increased tyrosinase activity by culturing for 4 days in EMEM + 0.5% FBS, supplemented with 500 μM cAMP, 100 μM PDE inhibitor IBMX and 100 μM Cu^{2+} (10 μg protein).

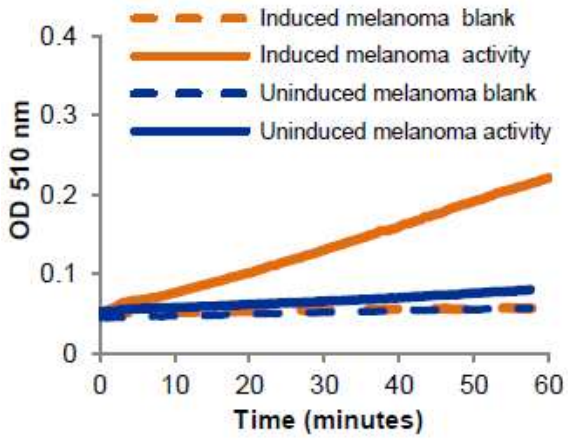
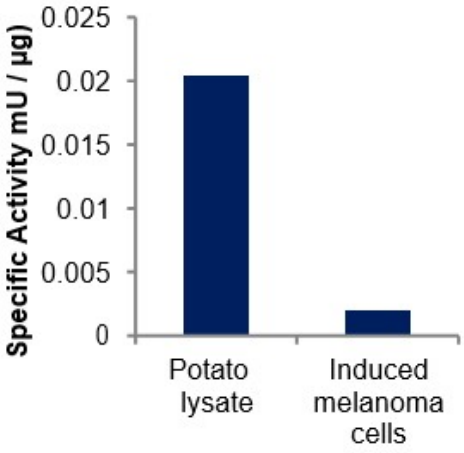


Figure 4.
Tyrosinase specific activity in potato lysate and melanoma cells.



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