Technical Bulletin

Tyrosinase Assay Kit

Catalogue number MAK550

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. 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 Assay Kit is a simple, one-step and reliable assay for monitoring tyrosinase activity with high sensitivity. The assay uses a colorless substrate solution that significantly increases its absorption at 510 nm upon reaction with tyrosinase. The increases in absorption at 510 nm is proportionate to the tyrosinase activity.

Components

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

- Tyrosinase Standard — 1 Vial
  Catalogue Number MAK550A
- Assay Buffer — 20 mL
  Catalogue Number MAK550B
- Tyrosinase Substrate — 1 Vial
  Catalogue Number MAK550C
- Tyrosinase Enhancer — 100 µL
  Catalogue Number MAK550D

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories.
- Spectrophotometric multiwell plate reader.
- Clear flat-bottom 96-well plates. Cell culture or tissue culture treated plates are not recommended.
- 1.5 mL microcentrifuge tubes.

Precautions and Disclaimer

For Research Use Only. Not for use 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 components at -20 °C.

Preparation Instructions

Briefly centrifuge small vials prior to opening.

Equilibrate to room temperature prior use.

Procedure

All Samples and Standards should be run in duplicate.

Preparation of Stock Solutions

1. Tyrosinase Stock Solution (2000 U/mL): Add 120 µL of Assay Buffer into the Tyrosinase Standard vial and mix well.
2. Tyrosinase Substrate Stock Solution (50X): Add 100 µL of purified water into the Tyrosinase Substrate vial and mix well.

Note: Store the unused Tyrosinase stock solution at -20 °C in single use aliquots.

Preparation of Standard Solution

1. Dilute the Tyrosinase stock solution (2000 U/mL) 1:5 using Assay Buffer to a final concentration of 400 U/mL to make Tyrosinase Standard solution (T1).
2. Perform 1:2 serial dilutions to produce the remaining serially diluted Tyrosinase Standards (T2-T7).

Note: With provided standard, 2 standard curves can be performed in duplicates if using at suggested concentrations.

Table 1.
Serial dilution of Tyrosinase Standard

Dilution	Tyrosinase Std (µL)	Serial Dilution Source	Assay Buffer Vol (µL)	Conc (U/ml)
T1	60	400 U/mL stock	240	400
T2	150	From T1	150	200
T3	150	From T2	150	100
T4	150	From T3	150	50
T5	150	From T4	150	25
T6	150	From T5	150	12.5
T7	150	From T6	150	6.25

Preparation of Working Solution

Make a 1:50 dilution by adding 20 µL Tyrosinase Substrate stock solution (50X) and 20 µL Tyrosinase Enhancer into 960 µL Assay Buffer and mix well.

Note: Tyrosinase Substrate working solution should be made immediately prior to use.

Assay Reaction

Add 50 µL of each standard, blank (Assay Buffer), and test samples into separate wells of a 96-well plate.

Add 50 µL of Tyrosinase Substrate working solution to each well containing a standard, blank, and test sample to make the total assay volume of 100 µL/well.

Incubate the reaction mixture at room temperature for 30 - 60 minutes.

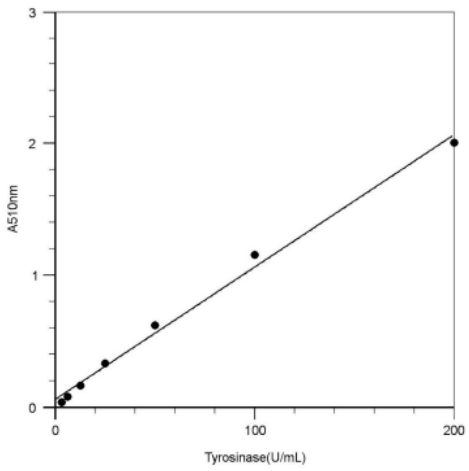
Measurement

Monitor the absorbance increase with an absorbance plate reader with path check on at OD of 510 nm.

Results

1. The reading (Absorbance) obtained from the blank standard well is used as a negative control.
2. Subtract the blank value from the standards to obtain the base-line corrected values.
3. Plot the standards' readings to obtain a standard curve and equation.
4. This equation can be used to calculate the Tyrosinase concentration of the samples.

Figure 1.
Typical Tyrosinase Standard Curve



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mak550pis Rev 11/23