Amplite™ Colorimetric Tyrosinase Assay Kit

Catalog number: 11311
Unit size: 100 Tests

Component	Storage	Amount
Component A: Tyrosinase Standard	Freeze (< -15 °C), Minimize light exposure	1 vial
Component B: Assay Buffer	Freeze (< -15 °C)	1 bottle (20 mL)
Component C: Tyrosinase Substrate	Freeze (< -15 °C), Minimize light exposure	1 vial
Component D: Tyrosinase Enhancer	Freeze (< -15 °C), Minimize light exposure	1 vial (100 µL)

OVERVIEW

Tyrosinase is of great interest to drug discovery, life science research, food industry and cosmetics industry since it plays an important role in the biosynthetic pathway of melanin. The development and screening of tyrosinase inhibitors has received great attentions to melanoma related illnesses. Tyrosinase levels and activity are highly upregulated in melanoma and considered to a reliable test to monitor melanoma related illnesses. AAT Bioquest has developed Amplite™ Colorimetric Tyrosinase Assay Kit. It is a simple, one-step and reliable assay for monitoring tyrosinase activity with very high sensitivity. The assay uses a proprietary substrate colorless solution that significantly increases its absorption at 510 nm upon reaction with tyrosinase. The increases in absorption at 510 nm is well correlated with tyrosinase activity. The assay kit is designed to be run with a microplate reader.

AT A GLANCE

Protocol Summary

1. Prepare and add standards and samples (50 µL)
2. Prepare and add Tyrosinase Substrate working solution to the standards and samples wells (50 µL)
3. Incubate the plate at room temperature for 30 to 60 minutes
4. Monitor the absorbance at 510 nm

Important Bring all the kit components at room temperature before starting the experiment.

KEY PARAMETERS

Absorbance microplate reader

Absorbance	510 nm
Recommended plate	White plate/Clear bottom

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

1. Tyrosinase stock solution (2000 U/mL)
Add 120 µL Assay Buffer (Component B) into Tyrosinase Standard (Component A) and mix well.

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

2. Tyrosinase Substrate stock solution (50X)
Add 100 µL ddH_2O into Assay Substrate (Component C) and mix well.

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

PREPARATION OF STANDARD SOLUTION

For convenience, use the Serial Dilution Planner:

https://www.aatbio.com/tools/serial-dilution/11311

Tyrosinase standard
Use Tyrosinase stock solution (2000 U/mL) and Assay Buffer to generate 400 U/mL final concentration of Tyrosinase Standard solution (T1). Then perform 1:2 serial dilutions to get remaining serially diluted Tyrosinase Standards (T2-T7). Note: The final in well concentration of the standards will be 2X. Note: With provided standard, 2 standard curves can be performed in duplicates if using at suggested concentrations.

PREPARATION OF WORKING SOLUTION

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

Note Tyrosinase Substrate working solution should be made immediately upon use. We recommend using working solution within several hours.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of Tyrosinase standards and test samples in a white-clear bottom 96- wells microplate. Tyrosinase standards (T1-T7= 400 to 6.25 U/mL), TS= Test Samples, BL= Blank samples

T1	T1	TS	TS
T2	T2		
T3	T3		
T4	T4		
T5	T5		
T6	T6		
T7	T7		
BL	BL		

The following protocol can be used as a guideline and should be optimized according to the needs.

1. Prepare the standards and test samples as per recommendations in assay buffer and add 50 µL of each in a microplate.
2. Add 50 µL Tyrosinase Substrate working solution to the wells of standards and samples.
3. Incubate the reaction at room temperature for 30 to 60 minutes.
4. Monitor the absorbance with an absorbance plate reader at 510 nm.

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (A510nm) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards' readings to obtain the base-line corrected values. Then, plot the standards' readings to obtain a standard curve and equation. This equation can be used to calculate Tyrosinase samples. We recommend using the Online Linear Regression Calculator which can be found at:

https://www.aatbio.com/tools/linear-logarithmic-semi-log-regression-online-calculator

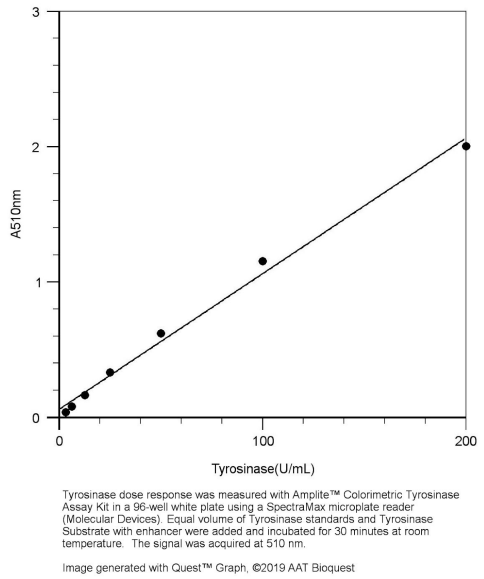

Figure 1. Tyrosinase dose response was measured with Amplite™ Colorimetric Tyrosinase Assay Kit in a 96-well white plate using a SpectraMax microplate reader (Molecular Devices). Equal volume of Tyrosinase standards and Tyrosinase Substrate with enhancer were added and incubated for 30 minutes at room temperature. The signal was acquired at 510 nm.

DISCLAIMER

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