

Amplite™ Fluorimetric Tyrosinase Assay Kit

 Catalog number: 11312
 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 Blue	Freeze (< -15 °C), Minimize light exposure	1 vial
Component D: DMSO	Freeze (< -15 °C)	1 vial (100 µL)

OVERVIEW

Tyrosinase is an enzyme expressed across a vast range of species from bacteria and fungi to animals. 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™ Fluorimetric 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 fluorogenic substrate that significantly increases its fluorescence intensity at 440 nm upon reaction with tyrosinase. The increases in fluorescence intensity at 440 nm is well correlated with tyrosinase activity. The assay kit is designed to be run with a fluorescence microplate reader.

AT A GLANCE

Protocol Summary

1. Prepare and add standards and samples (50 µL)
2. Prepare and add Tyrosinase Blue working solution to the standards and samples wells (50 µL)
3. Incubate the plate at room temperature for 60 to 120 minutes
4. Monitor the fluorescence intensity at Ex/Em= 340/440 nm

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

KEY PARAMETERS

Fluorescence microplate reader

Excitation	340 nm
Emission	440 nm
Cutoff	420 nm
Recommended plate	Solid black

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 a single use aliquots.

2. Tyrosinase Blue stock solution (100X):

Add 50 µL DMSO (Component D) into Tyrosinase Blue (Component C) and mix well. Note: Store the unused Tyrosinase Blue 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/11312>

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 concentrations of the standards in wells will be 2X. Note: With provided standards, 2 standard curves can be generated in duplicates if using at suggested concentrations.

PREPARATION OF WORKING SOLUTION

Tyrosinase Blue working solution

Make a 1:100 dilution by adding 5 µL Tyrosinase Blue stock solution (100X) to 1 mL Assay Buffer (Component B) and mix well.

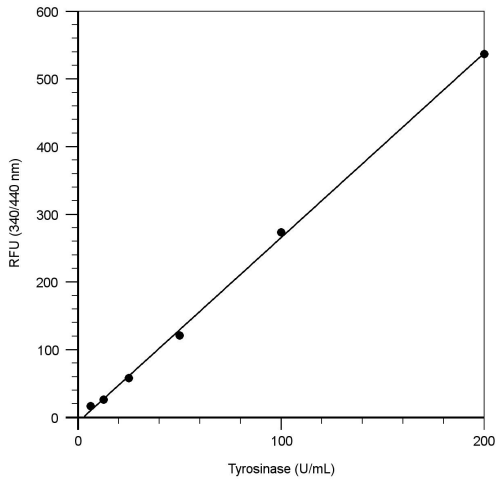
SAMPLE EXPERIMENTAL PROTOCOL

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		

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 Blue working solution to the wells of standards and samples.
 3. Incubate the reaction at 37 °C for 60 to 120 minutes.
- Note** The reaction can be kept up to 6 hours.
4. Monitor the fluorescence intensity with fluorescence plate reader at Ex/Em= 340/440 nm with cutoff= 420 nm.

EXAMPLE DATA ANALYSIS AND FIGURES



Tyrosinase dose response was measured with Amplitude™ Fluorimetric Tyrosinase Assay Kit in a 96-well black plate using a Gemini microplate reader (Molecular Devices). Equal volume of Tyrosinase standards and Tyrosinase Blue were added and incubated for 6 hours at 37 °C. The signal was acquired at Ex/Em = 340/440 nm (cut off at 420 nm).

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Figure 1. Tyrosinase dose response was measured with Amplitude™ Fluorimetric Tyrosinase Assay Kit in a 96-well black plate using a Gemini microplate reader (Molecular Devices). Equal volume of Tyrosinase standards and Tyrosinase Blue were added and incubated for 6 hours at 37 °C. The signal was acquired at Ex/Em = 340/440 nm (cut off at 420 nm).

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