Tyrosinase Assay Kit (Fluorometric)

Catalog Number KA6032

100 assays

Version: 01

Intended for research use only

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Introduction

Background

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.

Principle of the Assay

The Tyrosinase Assay Kit (Fluorometric) 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.

General Information

Materials Supplied

List of component

Component	Amount
Component A: Tyrosinase Standard	1 vial
Component B: Assay Buffer	20 mL
Component C: Tyrosinase Blue	1 vial
Component D: DMSO	100 µL

Storage Instruction

Component	Storage
Component A: Tyrosinase Standard	Freeze (<-15°C). Minimize light exposure.
Component B: Assay Buffer	Freeze (<-15°C).
Component C: Tyrosinase Blue	Freeze (<-15°C). Minimize light exposure.
Component D: DMSO	Freeze (<-15°C).

Materials Required but Not Supplied

Fluorescence microplate reader

Excitation: 340 nm

Emission: 440 nm

Cutoff: 420 nm

Recommended plate: Solid black

Precautions for Use

For research use only.

Assay Protocol

Reagent Preparation

✓ 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 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

- 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.

  The following protocol can be used to prepare a serial dilution of Tyrosinase standard with Assay buffer as the solvent. Values in the table indicate the reagent compositions per dilution.

Dilution	Tyrosinase standard (µL)	Assay buffer (µL)	[Tyrosinase standard] (U/mL)
1	60 (from 2000 U/mL stock)	240	400
2	150 (from dilution 1)	150	200
3	150 (from dilution 2)	150	100
4	150 (from dilution 3)	150	50
5	150 (from dilution 4)	150	25
6	150 (from dilution 5)	150	12.5
7	150 (from dilution 6)	150	6.25
Blank	0	150	0

1. Pipette 60 µL of 2000 U/mL Tyrosinase standard into 240 µL of Assay buffer. Mix well before continuing. Avoid generating bubbles.
2. Pipette 150 µL of dilution 1 (from step 1) into 150 µL of Assay buffer to create dilution 2. Mix well before continuing. Avoid generating bubbles.
3. Pipette 150 µL of dilution 2 (from step 2) into 150 µL of Assay buffer to create dilution 3. Mix

well before continuing. Avoid generating bubbles.

4. Pipette 150 µL of dilution 3 (from step 3) into 150 µL of Assay buffer to create dilution 4. Mix well before continuing. Avoid generating bubbles.
5. Pipette 150 µL of dilution 4 (from step 4) into 150 µL of Assay buffer to create dilution 5. Mix well before continuing. Avoid generating bubbles.
6. Pipette 150 µL of dilution 5 (from step 5) into 150 µL of Assay buffer to create dilution 6. Mix well before continuing. Avoid generating bubbles.
7. Pipette 150 µL of dilution 6 (from step 6) into 150 µL of Assay buffer to create dilution 7. Mix well before continuing. Avoid generating bubbles. Discard 150 µL from dilution 7 to obtain the correct volume for the final dilution.
8. Create a blank control using 150 µL of Assay buffer. This should be enough for 2 replicates.
9. Using the table in the protocol as a guide, pipette 50 µL of each standard into its corresponding well in the experimental microplate. Standards prepared with this protocol should be enough for 2 replicates. Use of a multi-channel pipette is highly recommended.

✓ 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.

Assay Procedure

1. Prepare the standards and test samples as per recommendations in assay buffer and add 50 µL of each in a microplate.

   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		

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.

- ✓ 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.

Data Analysis

Calculation of Results

The reading (RFU (340/440 nm)) 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.

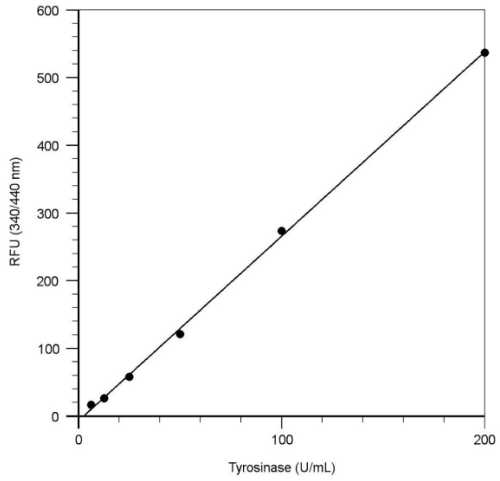

Figure 1. Tyrosinase dose response was measured with the Tyrosinase Assay Kit (Fluorometric) in a 96-well black plate using a Gemini microplate reader (Molecular Devices). Equal volume of Tyrosinase standards and Tyrosinase Blue were added and incubated for 6 hours at 37°C. The signal was acquired at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

Resources

Plate Layout

12								
11								
10								
9								
8								
7								
6								
5								
4								
3								
2								
1								
	A	B	C	D	E	F	G	H