Melanin Assay Kit (Fluorometric)

Catalog Number KA6030

100 assays

Version: 02

Intended for research use only

www.abnova.com

Table of Contents

Introduction

Background

Melanins have very diverse roles and functions in various organisms. Since melanins are an important biomarker, the accurate and sensitive determination of melanins has become a critical task for biomedical research and diagnostic applications. To address this unmet need, we have developed a robust fluorescence-based melanin assay.

Principle of the Assay

The Melanin Assay Kit (Fluorometric) uses a substrate that generates a fluorescent product upon reaction with melanins. Its fluorescence intensity is proportional to the amount of melanins in a sample. The Melanin Assay Kit (Fluorometric) provides a simple and effective method to measure melanin content in cells and other samples. The plate-based assay format is designed to use with a fluorescent microplate reader.

General Information

Materials Supplied

List of component

Component	Amount
Component A: Melanin Standard	1 vial
Component B: Assay Buffer	20 mL
Component C: Signal Enhancer	5 mL
Component D: DMSO	200 µL

Storage Instruction

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

Materials Required but Not Supplied

Fluorescence microplate reader

Excitation 470 nm

Emission 550 nm

Cutoff 515 nm

Recommended plate Solid black

Precautions for Use

For research use only.

Assay Protocol

Reagent Preparation

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.

✓ Preparation of stock solution

- Melanin stock solution

  Add 120 µL DMSO (Component D) into Melanin Standard (Component A) and mix well to generate a 5 mg/mL stock solution. Keep the mixture at room temperature for 10 minutes. Now the standard is ready to be used.

  Note: If you observe undissolved matter at the bottom, centrifuge the tube at 1000 rpm for 5 mins and take the supernatant and use that as a Melanin Standard solution.

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

✓ Preparation of standard solution

- Melanin standard

  Use Melanin stock solution and Assay Buffer to generate 500 µg/mL concentration of Melanin standard solution (M1). Then perform 1:2 serial dilutions to get remaining serially diluted Melanin standards (M2-M7).

  Note: The final in well concentration of the standards will be 1/2X.

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

Dilution	Melanin standard (µL)	Assay buffer (µL)	[Melanin standard] (µg/mL)
1	30 (from 5 mg/mL stock)	270	500
2	150 (from dilution 1)	150	250
3	150 (from dilution 2)	150	125
4	150 (from dilution 3)	150	62.5
5	150 (from dilution 4)	150	31.25
6	150 (from dilution 5)	150	15.63
7	150 (from dilution 6)	150	7.81
Blank	0	150	0

1. Pipette 30 µL of 5 mg/mL Melanin standard into 270 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.

Assay Procedure

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

   Table 1. Layout of Melanin standards and test samples in a solid black 96-wells microplate. Melanin standards (M1-M7= 500 to 7.81 µg/mL), TS= Test Samples, BL= Blank Samples

BL	BL	TS	TS
M1	M1	…	…
M2	M2	…	…
M3	M3		
M4	M4		
M5	M5		
M6	M6		
M7	M7		

2. Add 50 µL Signal Enhancer (Component C) to all the wells.
3. Incubate the reaction at room temperature for 30 to 60 minutes.
4. Monitor the fluorescence intensity with fluorescence plate reader at Ex/Em= 470/550 nm with cutoff= 515 nm.

✓ Summary

1. Prepare and add standards and samples (50 µL)
2. Add Signal Enhancer to the standards and wells (50 µL)
3. Incubate the plate at room temperature for 30 to 60 minutes
4. Monitor the fluorescence intensity at Ex/Em= 470/550 nm

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

Data Analysis

Calculation of Results

The reading (RFU (470/550 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 Melanin samples.

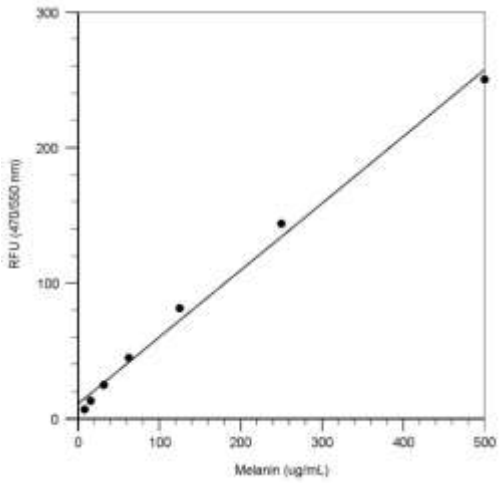

Figure 1. Melanin dose response was measured with the Melanin Assay Kit (Fluorometric) in a 96-well black plate using a Gemini microplate reader (Molecular Devices). Equal volume of melanin standards and Signal Enhancer were added and incubated for 45 minutes at room temperature. The signal was acquired at Ex/Em = 470/550 nm (cut off at 515 nm).

Resources

Plate Layout

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