Amplite™ Fluorimetric Melanin Assay Kit

Catalog number: 11310
Unit size: 100 Tests

Component	Storage	Amount
Component A: Melanin Standard	Freeze (< -15 °C), Minimize light exposure	1 vial
Component B: Assay Buffer	Freeze (< -15 °C)	1 bottle (20 mL)
Component C: Signal Enhancer	Freeze (< -15 °C)	1 bottle (5 mL)
Component D: DMSO	Freeze (< -15 °C)	1 vial (200 µL)

OVERVIEW

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. Amplite™ Fluorimetric Melanin Assay Kit 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. Amplite™ Fluorimetric Melanin Assay Kit 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.

AT A GLANCE

Protocol 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.

KEY PARAMETERS

Fluorescence microplate reader

Excitation	470 nm
Emission	550 nm
Cutoff	515 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.

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

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/11310

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.

SAMPLE EXPERIMENTAL PROTOCOL

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		

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 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.

EXAMPLE DATA ANALYSIS AND FIGURES

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. 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

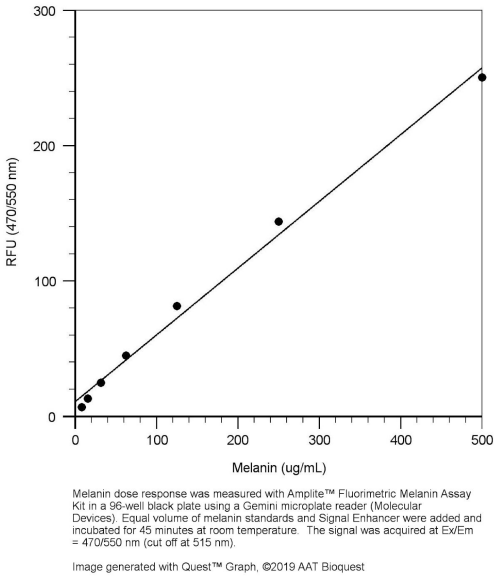

Figure 1. Melanin dose response was measured with Amplite™ Fluorimetric Melanin Assay Kit in a 96-well black plate using a Gemini microplate reader (Molecular Devices). Equal volume of melanin standards and Signal Enhancer were added and incubated for 45 minutes at room temperature. The signal was acquired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

DISCLAIMER

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