Technical Bulletin

Melanin Assay Kit

Catalogue number MAK557

Product Description

Melanin, a biological pigment found in various biological systems, exhibits a range of physiological properties. It is widely acknowledged for its ability to impact overall health through photoprotective and immunological actions. Additionally, melanin has antioxidant, anti-inflammatory, immunomodulatory, radioprotective, hepatic, gastrointestinal, neurological, and hypoglycemic effects.[1] The Melanin Assay Kit uses a substrate that generates a fluorescent product upon reaction with melanins. The fluorescence intensity is proportional to the amount of melanin in a sample.

The Melanin Assay Kit provides a simple and effective method to measure melanin content in cells and other biological samples.

Components

The kit is sufficient for 100 fluorometric assays in 96-well plates.

- Melanin Standard — 1 Vial
  Catalogue Number MAK557A
- Assay Buffer — 20 mL
  Catalogue Number MAK557B
- Signal Enhancer — 5 mL
  Catalogue Number MAK557C
- DMSO — 200 µL
  Catalogue Number MAK557D

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories.
- Fluorescence multiwell plate reader.
- Black, flat-bottom 96-well plates. Cell culture or tissue culture treated plates are not recommended.
- 1.5 mL microcentrifuge tubes

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet Ice. Store components at -20 °C.

Preparation Instructions

Briefly centrifuge small vials prior to opening.

Equilibrate to room temperature prior to use.

Procedure

All Samples and Standards should be run in duplicates.

Preparation of Melanin Stock Solution

Add 120 µL DMSO into the Melanin Standard vial and mix well to generate a 5 mg/mL stock solution. Keep the mixture at room temperature for 10 minutes before use.

Note: If you observe undissolved matter at the bottom, centrifuge the tube at 1000 rpm for 5 minutes and use the supernatant as the Melanin stock solution. Store any unused Melanin stock solution at -20 °C in single use aliquots.

Preparation of Melanin Standard Reaction

1. Use Melanin stock solution and Assay Buffer to generate 500 µg/mL concentration of Melanin standard solution (M1).
2. Perform 1:2 serial dilutions to produce the remaining serially diluted Melanin standards (M2-M7) as shown in Table 1.

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

Table 1.

Dilution of Melanin Standards

Dilution	Melanin Standard Volume (µL)	Serial Dilution Source	Assay Buffer Volume(µL)	Conc (µg/mL)
M1	30	5mg/mL Stock	270	500
M2	150	From M1	150	250
M3	150	From M2	150	125
M4	150	From M3	150	62.5
M5	150	From M4	150	31.25
M6	150	From M5	150	15.63
M7	150	From M6	150	7.81

Assay Reaction

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 to all the wells.
3. Incubate the reaction mixture at room temperature for 30 to 60 minutes.

Measurement

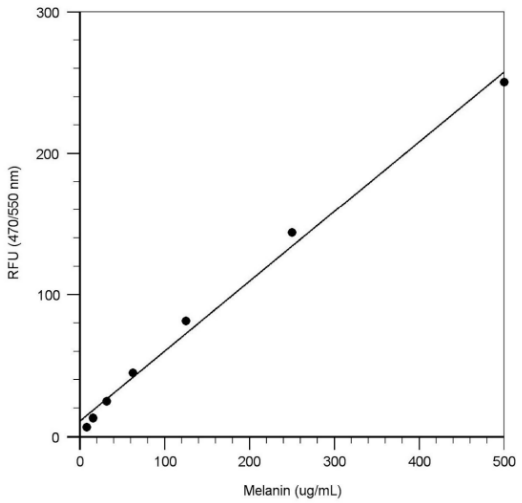
Monitor the fluorescence increase with a fluorescence plate reader at $\lambda_{Ex}/\lambda_{Em}$ = 470/550 nm with cutoff at 515 nm.

Results

1. The reading (RFU) obtained from the blank standard well is used as a negative control.
2. Subtract the blank value from the standards' readings to obtain the base-line corrected values.
3. Plot the standards readings to obtain the standard curve.
4. The concentration of melanin in the samples may be determined from the standard curve.

Figure 1.

Typical Melanin Standard Curve



References

1. ElObeid A.S., *et al*., Pharmacological Properties of Melanin and its Function in Health. *Basic Clin Pharmacol Toxicol,* 120: 515-522, (2017)

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