Apr 07, 2023

Chemical degradation and determination of pheomelanin and eumelanin markers

DOI
dx.doi.org/10.17504/protocols.io.36wgqjz43vk5/v1

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DOI: https://dx.doi.org/10.17504/protocols.io.36wgqjz43vk5/v1

Protocol Citation: Kazumasa Wakamatsu 2023. Chemical degradation and determination of pheomelanin and eumelanin markers. **protocols.io https://dx.doi.org/10.17504/protocols.io.36wgqjz43vk5/v1**

Protocol status: Working
We use this protocol and it's working

Created: April 07, 2023

Last Modified: December 13, 2024

Protocol Integer ID: 80142

Keywords: ASAPCRN, eumelanin marker, pheomelanin, eumelanin, high performance liquid chromatography, chemical degradation, detection of marker, chemical oxidation, involving chemical oxidation

Funders Acknowledgements:
From cancer associations to altered immunity in the pathogenesis of Parkinson's disease
Grant ID: ASAP-000312

Abstract

This is protocol involving chemical oxidation and reduction methods followed by high performance liquid chromatography (HPLC) detection of markers for pheomelanin and eumelanin.

Troubleshooting

1 Homogenize samples in water with Ten-Broeck glass homogenizer at a concentration of 10 mg/mL (if samples were < 5 mg, use 0.5 mL of water).

2 100 µL aliquots are then subjected to the chemical reactions.

3 Oxidation: Oxidize samples with 1.5% H2O2/K2CO3. After termination of reaction, leave mixtures for 20 hours at 25ºC (inducing secondary production of PTCA, PDCA, and TTCA)

4 Reduction: Heat samples with 57% HI in presence of H3PO2 at 130ºC for 20 hours. Analyze products, 4-AHP and 4-AHPEA

5 Levels of 4-AHP, the degradative product of DOPA pheomelanin, and 4-AHPEA, the degradative product of DA pheomelanin were analyzed by HPLC-ECD.

Protocol references

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