



APR 06, 2023

OPEN  ACCESS

**DOI:**  
[dx.doi.org/10.17504/protocols.io.36wgqjz43vk5/v1](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>

**License:** This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

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

**Created:** Apr 06, 2023

**Last Modified:** Apr 06, 2023

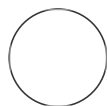
**PROTOCOL integer ID:**  
 80142

**Keywords:** ASAPCRN

## Chemical degradation and determination of pheomelanin and eumelanin markers

Kazumasa Wakamatsu<sup>1</sup>

<sup>1</sup>Institute for Melanin Chemistry, Fujita Health University, Toyoake, Japan



Daniel Choo

### ABSTRACT

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

**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% H<sub>2</sub>O<sub>2</sub>/K<sub>2</sub>CO<sub>3</sub>. After termination of reaction, leave mixtures for 20 hours at 25°C (inducing secondary production of PTCA, PDCA, and TTCA)
- 4** Reduction: Heat samples with 57% HI in presence of H<sub>3</sub>PO<sub>2</sub> at 130°C 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.