# Synthesis and preparation of DOPA pheomelanin and eumelanin

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We use this protocol and it's working

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## ABSTRACT

This is the protocol for synthesizing and preparing DOPA pheomelanin and DOPA eumelanin using tyrosinase.

## ATTACHMENTS

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1 As precursor(s) of melanin, 1) l-dopa, 2) DHI, 3) a mixture of DHI and DHICA, 4) DHICA, 5) a mixture of l-dopa and l-cysteine, and 6) 5SCD in the presence of l-dopa is used.

2 To a solution of the precursor(s) (1 mmol) in 0.1 M sodium phosphate buffer, pH 6.8 (98 ml), add a solution of mushroom tyrosinase in the same buffer (2 ml) at 25˚C.

2.1 The amount of mushroom tyrosinase (Sigma-Aldrich, 4270 U/mg) is either 50,000 U or 100,000 U.

3 Carry out oxidation with vigorous (maximal, but avoiding splashing) stirring in a 500 ml Erlenmeyer flask.

4 Stop oxidation (1-4) at 4 h by adding 6 M HCl (2 ml) to make pH 1 and keep the mixture at 4˚C overnight to ensure complete precipitation of the pigment.

5 Collect the precipitate by centrifugation at 3,000 rpm for 10 min and wash with 0.1 M HCl (40 ml x 3).

6 For the preparation of pheomelanins (5 and 6), acidify the oxidation mixture with acetic acid (3 ml) to make pH 3, and wash the precipitate with 1% acetic acid (40 ml x 3).

7 Dry the melanin powder by lyophilization and equilibrate with moisture in a desiccator with a saturated $CaCl_2$ solution.

8 Prior to treatment, suspend the synthetic DOPA pheomelanin (DOPA:Cys = 1:1) and DOPA eumelanin (DOPA:Cys = 1:0) in sterile PBS and sonicate overnight.