APR 21, 2023

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.3byl4j4xrlo5/v1

Protocol Citation: matthew.morgan 2023. Spectrophotometric Quantification of Betacyanins in Plant Tissue Produced from the RUBY Reporter Gene. **protocols.io** https://dx.doi.org/10.17504/protocols.io.3byl4j4xrlo5/v1

MANUSCRIPT CITATION:

Protocol status: In development
We are still developing and optimizing this protocol

Created: Apr 12, 2023

Last Modified: Apr 21, 2023

PROTOCOL integer ID: 80365

Spectrophotometric Quantification of Betacyanins in Plant Tissue Produced from the RUBY Reporter Gene

matthew.morgan[1]

[1]Adelaide University

matthew.morgan

ABSTRACT

RUBY is a visual reporter gene that produces beatnin, a betacyanin that appears red to violet in colour (He et al., 2020). To assay betanin (and other betalains) a spectrophotometric approach has been employed, as seen in Stintzing et al. (2003) and Grützner et al. (2021).

MATERIALS

- 2 ml micro-centrifuge tubes
- Mini pestle
- Methanol
- Ascorbic acid
- Formic acid
- Cuvettes/96 well plates
- Spectrophotometer

SAFETY WARNINGS

Methanol SDS

Sample collection

1 Punch out leaf discs (as many as possible in the target area) of areas expressing betanin.

2 Put leaf discs in 2 ml micro-centrifuge tubes and snap freeze in liquid nitrogen then store in -80 freezer until required.

Sample Preparation

3 Take 20-50 mg of frozen tissue and add to 2 ml tubes. Keep on ice.

4 Grind down tissue using plastic micro-pestle and add methanol buffer (50% methanol, 1 mM ascorbic acid, 0.5% formic acid) at 10% w/v - i.e. for 35 mg of tissue add 350 ul methanol buffer.

5 Dilute samples with mili-Q so that absorption values fall within 0.8 - 1.0 (as previously calculated by Stintzing et al. 2003). Grützner et al. (2021) found that a 12 fold dilution performed well but this could differ.

Spectrophotometric Quantifications

6 Put cuvettes or 96 well plates into spectophotometer and read absorbtion at 538 nm (Stintzing et al. 2003).

7 Using the formula BC = (A * DF * MW * 1000) / (ε x L) calculate the concentration of betacyanins.

BC = betanin content (mg/L) **A** = absorbance (538 nm) **DF** = dilution factor **MW** = molecular weight (550.47 g/mol) **L** = cuvette path length **ε** = molar extinction coefficient (60, 000 L/(mool cm)