

DMBA Assay for Phlorotannin

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Contents

| | |
|-------------------------|---|
| Introduction | 1 |
| Reagents..... | 1 |
| Original Method | 1 |
| Microscale Method | 2 |

Introduction

Phlorotannins are found in brown algae. They are based on phloroglucinol-type monomers. In a reaction similar to the reaction of vanillin with the meta-substituted ring of flavanoids, phlorotannins react with 2,4 dimethoxy benzaldehyde in glacial acetic acid to form a red conjugate.

The method given here is the original method (Stern J. L.; Hagerman, A. E.; Steinberg, P. D.; Winter, F. C.; Estes, J. A. A new assay for quantifying brown algal phlorotannins and comparisons to previous methods. *J. Chem. Ecol.* 1996, 22, 1273-1293) and also a microscale method.

This reaction can be used to determine phlorotannin in phlorotannin-protein precipitates. Because of the acetic acid fumes, as many steps as possible should be done in a chemical fume hood. Tubes and water baths should be covered to minimize release of fumes into the surroundings.

Reagents

- Standard tannin dissolved in methanol at 10 mg/mL. Prepare fresh daily. (Or use methanol extract of algae of interest).
- 16% HCl in glacial acetic acid: 16 mL conc HCl made up to 100 mL with glacial acetic acid. Stable at room temperature.
- DMBA: 2.0 g of 2,4-dimethoxy benzaldehyde (Sigma D 3269) made up to 100 mL with glacial acetic acid. Prepare fresh daily. Prepare only the amount needed. Will require 1.25 mL DMBA per sample (original method) or 700 uL per sample (microscale method).

Original Method

1. Bring water bath to 30 °C.
2. Dispense samples (0-20 uL of standard tannin solution or plant extract) into conical 15 mL glass tubes. Add methanol to make volume to 20 uL.
3. Add 10 uL N,N-dimethyl formamide (DMF) to each sample.
4. Add 1.25 mL 16% HCl to each sample. Mix.
5. At exactly 1 min intervals, add 1.25 mL of DMBA reagent to each sample, mix, and place sample in 30 °C bath. Cover samples.

6. Exactly 60 min after addition to first sample, start reading absorbance at 510 nm at 1 min intervals.
7. Spec should be zeroed on 16% HCl reagent.

Microscale Method

This method has the advantage of using less reagent and sample, and is done in the snap-top microfuge tubes so that fumes are contained very well. It is linear to about 70 ug of Ecklonia tannin.

1. Dispense samples (0-10 uL of 5 ug/uL tannin solution or plant extract) into 1.5 mL microfuge tubes.
2. Add methanol to make volume to 10 uL.
3. Add 10 uL DMF to each sample.
4. Add 700 uL 16% HCl to each sample. Mix.
5. At exactly 1 min intervals, add 700 uL DMBA reagent to each sample, cap, vortex vigorously, and place sample in 30 °C water bath.
6. Exactly 60 min after addition to first sample, start reading absorbance at 510 nm at 1 min intervals, using microcuvette.
7. Spec should be zeroed with 16% HCl solution.