

## MALDI-TOF Analysis of Debranched Starch

### Procedure

- 1) Resuspend purified starch to a concentration of 2–5 mg/ml in ddH<sub>2</sub>O. Gel the starch suspension by heating in a boiling water bath for 1 h with occasional agitation.
- 2) Add sodium acetate to 50 mM from a 1 M stock solution. Add sodium azide to 0.05% from a 10% stock solution. Add 500 units isoamylase per mg of starch. Mix well by vortexing.
- 3) Incubate overnight (16-24 h) at 37°C.
- 4) Inactivate the isoamylase by boiling 5 min.
- 5) Centrifuge 10 min at room temperature.
- 6) Remove a portion of the supernatant for analysis. Frequently it must be diluted ~5-fold prior to MALDI-TOF analysis.
- 7) Mix debranched starch 1:1 with the THAP matrix solution and spot on a MALDI target.
- 8) Acquire spectrum and perform baseline correction over the range  $m/z = 700-8000$  (DP ~6–50).
- 9) Use an analysis program to measure the area under the peaks. Rescale each spectrum relative to the largest peak to make comparisons between samples.

### Solutions

<p><b>1 M Sodium Acetate</b> 13.61 g sodium acetate dissolved in ~80 ml ddH<sub>2</sub>O. Titrate to pH 4.5 with 1 M NaOH. Add ddH<sub>2</sub>O to bring volume to 100 ml.</p>	<p><b>Isoamylase Solution</b> Isoamylase (Sigma I-2758) diluted in 25 mM sodium acetate pH 4.5 plus 0.05% sodium azide to a final concentration of 1000 units/<math>\mu</math>l.  Store at 4°C.</p>
<p><b>10% Sodium Azide [w/v]</b> 1 g Sodium Azide. dd H<sub>2</sub>O to a final volume of 10 ml.</p>	<p><b>THAP Matrix Solution</b> 0.1 M 2,4,6-trihydroxyacetophenone (Aldrich T6,460-2) in methanol.</p>

### Procedure adapted from:

**Nishi, A., Nakamura, Y., Tanaka, N., and Satoh, H.** (2001). Biochemical and genetic analysis of the effects of amylose-extender mutation in rice endosperm. *Plant Physiol.* **127**, 459-472.

**Broberg, S., Koch, K., Andersson, R., and Kenne, L.** (2000). A comparison between MALDI-TOF mass spectrometry and HPAEC-PAD analysis of debranched starch. *Carbohydr. Polym.* **43**, 285-289.