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# Iodine staining of starch to determine amylose percentage

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## ABSTRACT

Starch is well known to complex with iodine producing strong absorbance between 500 and 750 nm. Two structures that compose structure, amylose and amylopectin complex with iodine differently resulting in distinct absorption spectra. The staining is blue for amylose, brown for amylopectin, and yellow for soluble polymers of glucose (ex. glycogen).

Measuring iodine-stained samples at 525 nm and 700 nm allows for the calculation of the percent amylose.

## THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Hostettler, C., Kölling, K., Santelia, D., Streb, S., Kötting, O., and Zeeman, S. C. (2011) Analysis of starch metabolism in chloroplasts. *Methods Mol. Biol.* 775, 387–410.

## PROTOCOL CITATION

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<https://protocols.io/view/iodine-staining-of-starch-to-determine-amylose-per-bs2qngdw>

## MANUSCRIPT CITATION please remember to cite the following publication along with this protocol

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## MATERIALS TEXT

Spectrophotometer capable of measuring absorbance between 500 and 750 nm.

Water

Cuvettes (plastic or quartz)

A warm water bath at 95 C

0.34% (w/v) I<sub>2</sub> and 0.68% (w/v) KI solution (need 1 mL per sample or control)

## SAFETY WARNINGS

Wear goggles, gloves, and be careful handling the warmed starch samples. The tops of plastic tubes may pop

when handled.  
Be careful not to burn your fingers in the water bath.  
Iodine solution can stain skin and clothing

#### BEFORE STARTING

Obtain the starch sample.

#### Preparing starch for spectroscopic measurement

1 Obtain soluble starch or starch granule sample in sterile water

2 Warm sample to **95 °C** in a water bath for **00:15:00**

15m

Starch granules will gelatinize under these conditions. Do not cool the samples otherwise staining may not work efficiently

3 Add **20 µl** of starch solution to a cuvette.

Plastic cuvettes will become stained with iodine solution and cannot be reused.

4 Add **980 µl** of Iodine solution. Mix by inverting.

#### Measuring absorbance of iodine stained samples

5 Measure absorbance at 525 nm and 700 nm. Record values in the table. Add more columns for each new sample and edit the column name to indicate the sample.

A	B
Wavelength	Absorbance
525 nm	
700 nm	

6 Add **20 µl** of water or whatever buffer the starch is dissolved in to a new cuvette and then add **980 µl** of the iodine solution. Mix by inverting.

7 Measure absorbance at 525 nm and 700 nm.

A	B
Wavelength	Absorbance
525 nm	
700 nm	

#### Calculating percentage amylose

8 Subtract the absorbance of water-iodine measured in Step 7 from the absorbance for the sample(s) containing starch.

9 The percentage of the sample that is amylose is determined from the equation below:

$$\text{percent amylose} = \frac{3.039 - (7.154 \times \frac{OD_{700}}{OD_{525}})}{(3.048 \times \frac{OD_{700}}{OD_{525}}) - 19.192}$$

10 Record your calculations in the table below.

A	B
Sample name	% amylose