

EnzyChrom™ Starch Assay Kit (Cat# ESTA-100)

Quantitative Colorimetric Starch Determination at 585nm

DESCRIPTION

STARCH, chemical formula $(C_6H_{10}O_5)_n$, is a polysaccharide carbohydrate consisting of a large number of glucose units joined together by glycosidic bonds. All plant seeds and tubers contain starch present in the form of amylose and amylopectin. Starch is the most consumed polysaccharide in the human diet. Some starches are digested very quickly, and cause a rapid and large rise in blood sugar. Others are digested more slowly, and some starch, called resistant starch, is not digested in the small intestine at all, and thus causes little or no blood sugar rise.

Simple, direct and automation-ready procedures for measuring starch concentrations find wide applications in food, biochemical research and drug discovery. BioAssay Systems' starch assay uses α -amylase/amyloglucosidase hydrolysis reagent that rapidly breaks down starch into glucose, which is determined with our proprietary colorimetric glucose assay reagent. This simple convenient assay is carried out at room temperature and takes 45 min.

KEY FEATURES

Use as little as 10 μ L samples. Linear detection range 0.6 to 50 μ g/mL (6 to 500 ng) starch.

KIT CONTENTS

Assay Buffer: 20 mL
Enzyme A: 120 μ L
Enzyme B: 120 μ L
Standard: 50 μ L 50 mg/mL Standard
Detection Reagent: 20 mL

Storage conditions. The kit is shipped on dry ice. Store Standard and detection Reagent at 4°C and others at -20°C. Shelf life of three months after receipt.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

Sample Preparation

Soluble Starch. Grind up 5-10 mg sample, wash off any free glucose and small oligosaccharides with 1 ml 90% ethanol, warm to 60°C for 5 minutes with occasional vortexing. Centrifuge at 10,000g for 2 minutes. Decant the supernatant. Repeat the wash twice. Remove ethanol.

Soluble starch in the pellet is extracted with 1 mL H₂O incubated in a boiling water bath for 5 minutes. Spin 10,000g for 2 minutes. The supernatant is soluble starch and resistant starch is in the insoluble pellet.

Resistant Starch. After extracting soluble starch, extract the water insoluble pellet with 0.2 mL DMSO and heat in boiling water bath for 5 minutes. Dilute sample 1:100 in H₂O prior to assay.

Alternatively, resistant starch can be extracted with KOH/H₃PO₄ or KOH/acetate method [1].

Reagent Preparation: Bring all reagents to room temperature. Keep thawed enzyme tubes on ice or in refrigerator during the experiment.

Assay Procedure

1. Dilute standard by mixing 5 μ L Standard with 5 mL H₂O to give 50 μ g/mL standard. Transfer 10 μ L H₂O (*Blank*) and 10 μ L diluted standard into wells of a clear flat-bottom 96-well plate. Transfer 10 μ L samples into separate wells.

2. For each assay well, prepare hydrolysis reagent by mixing 42 μ L Assay Buffer, 1 μ L Enzyme A and 1 μ L Enzyme B. Add 40 μ L reagent per well. Tap plate to mix and incubate for 15 min at room temperature.

3. Add 150 μ L Detection Reagent per well. Tap plate to mix and incubate 30 min. Measure optical density at 585nm (540-610nm).

Note: if Sample OD values are higher than that of the standard, dilute sample in water, repeat assay and multiply results by the dilution factor.

CALCULATION

The starch concentration of Sample is calculated as

$$[\text{Starch}] = \frac{OD_{\text{SAMPLE}} - OD_{\text{H}_2\text{O}}}{OD_{\text{STANDARD}} - OD_{\text{H}_2\text{O}}} \times 50 \text{ (}\mu\text{g/mL)}$$

OD_{SAMPLE}, OD_{STANDARD} and OD_{H₂O} are optical density values of the sample, the 50 μ g/mL Standard and H₂O.

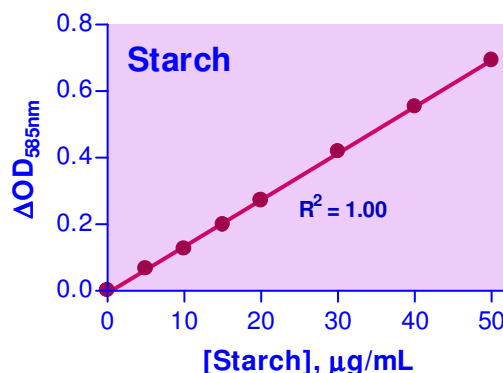
GENERAL CONSIDERATIONS

1. This assay is based on a kinetic reaction, the use of a multi-channel pipettor for adding the hydrolysis and detection reagent is recommended.

2. Interference. Several chemicals are known to interfere and should be avoided in sample preparation. These include ascorbic acid, EDTA, heparin, NP-40 (>0.6%), SDS (>0.12%) and Tris (>8 mM).

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipeting devices, centrifuge tubes, clear flat bottom 96-well plates and plate reader.



Standard Curve in 96-well plate assay

LITERATURE

- Official Methods of Analysis of AOAC International, 17th Edition. Edited by William Horwitz. AOAC International (2000).
- Chow PS, Landhäuser SM. (2004). A method for routine measurements of total sugar and starch content in woody plant tissues. *Tree Physiol.* 24(10):1129-36.
- Grant GA, Frison SL, Yeung J, Vasanthan T, Sporns P. (2003). Comparison of MALDI-TOF mass spectrometric to enzyme colorimetric quantification of glucose from enzyme-hydrolyzed starch. *J Agric Food Chem.* 51(21):6137-44.
- McCleary BV, Monaghan DA (2002). Measurement of resistant starch. *J AOAC Int.* 85(3):665-75.