EnzyChrom™ Starch Assay Kit (Cat# E2ST-100)

Quantitative Colorimetric/Fluorimetric Starch Determination

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 research and drug discovery. BioAssay Systems' starch uses a single Working Reagent that combines the enzymatic break down of starch and the detection of glucose in one step. The color intensity of the reaction product at 570 nm or fluorescence intensity at $\lambda_{ex/em}$ = 530/585 nm is directly proportional to the starch concentration in the sample. This simple convenient assay is carried out at room temperature and takes only 30 min.

KEY FEATURES

Use as little as 10 µL samples. Linear detection range: 2 to 200 µg/mL starch for colorimetric assays and 0.2 to 20 µg/mL for fluorimetric assays.

KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

Assay Buffer:	12 mL	**Dye Reagent:**	120 µL
Enzyme A:	Dried	**Standard:**	50 µL 50 mg/mL
Enzyme B:	120 µL		

Storage conditions. The kit is shipped on ice. Store all components at -20°C upon receiving. Shelf life: 6 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

Reagent Preparation:

Reconstitute Enzyme A by adding 120 µL Assay Buffer to the Enzyme A tube. Make sure Enzyme A is fully dissolved by pipetting up and down. Store reconstituted Enzyme A at -20°C and use within 1 month.

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 min with occasional vortexing. Centrifuge at 10,000g for 2 min. Decant the supernatant. Repeat the wash twice. Remove ethanol.

Soluble starch in the pellet is extracted with 1 mL H_2O incubated in a boiling water bath for 5 min. Spin 10,000g for 2 min. 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 min. Dilute sample 1:100 in H_2O prior to assay. Alternatively, resistant starch can be extracted with KOH/H_3PO_4 or KOH/acetate method [1].

Colorimetric Procedure:

1. Equilibrate all components to room temperature. During experiment, keep thawed enzymes in a refrigerator or on ice.
2. *Standards and Samples*: Dilute standard by mixing 5 µL Standard with 1.245 mL dH_2O to give 200 µg/mL standard. Dilute 200 µg/mL standard in dH_2O as follows.

No	200 µg/mL STD + H_2O	Vol (µL)	Starch (µg/ml)
1	200 µL + 0 µL	200	200
2	150 µL + 50 µL	200	150
3	100 µL + 100 µL	200	100
4	50 µL + 150 µL	200	50
5	0 µL + 200 µL	200	0

Transfer 10 µL Standards and Samples into separate wells of a clear flat-bottom microplate. If the sample contains glucose, transfer an additional 10 µL sample to another well for the Sample Blank.

3. *Working Reagent*. For each Standard and Sample well, Prepare *Working Reagent* by mixing 90 µL Assay Buffer, 1 µL Enzyme A, 1 µL Enzyme B, and 1 µL Dye Reagent. For Sample Blank wells, prepare *Blank Working Reagent* by mixing 90 µL Assay Buffer, 1 µL Enzyme B, and 1 µL Dye Reagent (*No Enzyme A*). Transfer 90 µL Working Reagent into each Standard and Sample well. Transfer 90 µL Blank Working Reagent to each Sample Blank well. Tap plate to mix.
4. Incubate 30 min at room temperature. Read optical density at 570 nm (550-585 nm).

Fluorimetric Procedure:

For fluorimetric assays, the linear detection range is 0.2 to 20 µg/mL starch. Follow steps 1-3 of the colorimetric procedure, but prepare 0, 5, 10, 15 and 20 µg/mL Standards and use a black flat-bottom microplate. Incubate 30 min at room temperature and read fluorescence at $\lambda_{ex/em}$ = 530/585 nm.

CALCULATION

Subtract Blank reading (OD_{570nm} or fluorescence intensity) from the standard reading values and plot the ΔOD or ΔF against standard concentrations. Determine the slope and calculate the starch concentration of the sample.

$$\text{Starch} = \frac{R_{SAMPLE} - R_{BLANK}}{\text{Slope}}\ \mu g/mL$$

R_{SAMPLE} and R_{BLANK} are the OD_{570nm} or fluorescence intensity values of the sample and blank (water, or sample blank).

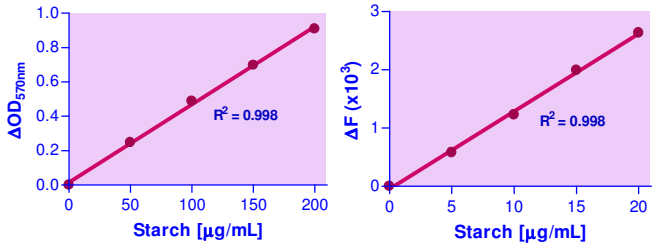
GENERAL CONSIDERATIONS

1. This assay is based on a kinetic reaction, the use of a multi-channel pipettor for adding the working reagent is recommended.
2. SH-group containing reagents (e.g., DTT, β-mercaptoethanol) may interfere with this assay and should be avoided in sample preparation.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices, centrifuge tubes, clear flat-bottom uncoated 96-well plates (e.g. VWR cat# 82050-760), optical density plate reader; black flat-bottom uncoated 96-well plates (e.g. VWR cat# 82050-676), fluorescence plate reader.

Starch Standard Curves



96-well colorimetric assay ***96-well fluorimetric assay***

PUBLICATIONS

1. South, P. F., Cavanagh, A. P., Liu, H. W., & Ort, D. R. (2019). Synthetic glycolate metabolism pathways stimulate crop growth and productivity in the field. Science, 363(6422), eaat9077.
2. Liu, F., et al. (2020). An invasive beetle-fungus complex is maintained by fungal nutritional-compensation mediated by bacterial volatiles. The ISME Journal, 14(11), 2829-2842.
3. Gardner RD et al (2013). Comparison of CO(2) and bicarbonate as inorganic carbon sources for triacylglycerol and starch accumulation in Chlamydomonas reinhardtii. Biotechnol Bioeng. 110(1):87-96.