CREATIVE BIOMART®
Assay Kit

Starch Assay Kit

Product Information

Cat

Kit-0805

Cat.No.

Kit-0805

Product Overview

Simple, direct and automation-ready procedures for measuring starch concentrations find wide applications in research and drug discovery. Our 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 λex/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.

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.

Applications

For quantitative determination of starch and evaluation of drug effects on starch metabolism.

Notes

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

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Storage

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

Size

100 tests

Kit Components

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

Materials Required but Not Supplied

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

Detection method OD570nm, or FL530/585nm

Compatible Sample Types

Biological, agriculture, food etc

Features & Benefits

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.

Preparation

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.

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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.

Assay Protocol

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 dH2O to give 200 µg/mL standard. Dilute standard in dH2O as follows.
No 200 µg/mL STD + H2O 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 standard and samples into separate wells of a clear flat-bottom microplate.
3. Working Reagent. For each reaction well, mix 90 µL Assay Buffer, 1 µL Enzyme A, 1 µL Enzyme B and 1 µL Dye Reagent in a clean tube.
Transfer 90 µL Working Reagent into each reaction well. Tap plate to mix.
4. Incubate 30 min at room temperature. Read optical density at 570 nm (550-585 nm).

Fluorimetric Procedure:

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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 Standard and use a black flat-bottom microplate. Incubate 30 min at room temperature and read fluorescence at λex = 530 nm and λem = 585 nm.

Assay time

30 min

Analysis

Subtract Blank reading (OD570nm 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.
Starch = (RSAMPLE - RBLANK)/ Slope = µg/mL
RSAMPLE and RBLANK are the OD570nm or fluorescence intensity values of the sample and blank (water, or sample blank, see below).

Sensitivity

2 µg/mL

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