Starch

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1022

(Version 2.1D)

Detection and Quantification of Starch Content in food, feed, plant andcereal products.

For research use only. Not for diagnostic or therapeutic procedures.

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I. INTRODUCTION

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.

This procedure initiates by pretreatment with 80% ethanol to remove the soluble sugar,followed bygelatinization, liquefaction and hydrolysis of dextrin to glucose. The intensity of the product color, measured at 550 nm, is proportional to the starch in the sample.This procedure is not suitable for samples containing high levels of resistant starch.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Reaction Buffer	13 mlx 1	4 °C
Enzyme I	Powder x 1	-20 °C, keep in dark
Enzyme II	Powder x 2	-20 °C, keep in dark
Enzyme III	Powder x 1	-20 °C, keep in dark
EnzymeDiluent	10 ml x 1	4 °C
Dye Reagent	Powder x1	4 °C, keep in dark
Standard	Powder x1	4 °C
Positive Control	Powder x 1	4 °C
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III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 550 nm
2. Distilled water
3. Boiling water bath and 50°Cwater bath
4. Pipettor, multi-channel pipettor
5. Pipette tips
6. Mortar
7. Ice
8. Centrifuge
9. Timer
10. 80% v/v aqueous ethanol

IV. REAGENT PREPARATION

Enzyme I: Dissolvein3 ml Enzyme Diluent before use.Store at -20 °C for 2weeks.

Enzyme II: Briefly centrifuge prior to opening. Dissolveeach Enzyme II vial in1.25 ml Enzyme Diluent before use.Store at 4°C for 3-7 days.

Enzyme III: Briefly centrifuge prior to opening. Dissolvein1ml Reaction Buffer before use.Store at 4°C for 3-7 days.

Dye Reagent:Add5 ml distilled waterinto the bottle, dissolve it absolutely before use.Store at 4°C for 3-7 days.

Standard: Briefly centrifuge prior to opening. Add 1 ml distilled water to dissolve before use.Then add 0.1 ml into 0.9 ml distilled water, mix, the concentration will be 0.5mg/ml, store at 4 °C for 2 weeksafter reconstitution.Perform 2-fold serial dilutions of the top standardsolution using distilled waterto make the standard curve. The concentration of standard curve could be 0.5/0.25/0.125/0.0625/0.0312/0.0156 mg/ml.

Positive Control: Add 5 ml distilled water to dissolve before use.Fasten downin case moisture loss and boil in a boiling water bath for 1-2 minutes.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

V. SAMPLE PREPARATION

1.For samplesNOT rich in glucose and/or maltodextrins (e.g.Wheat flour)

Weigh50mg sample into a 10ml tube. Add 5ml distilled water to wet the sample and stir the tube by vertexing. Fasten downin case moisture loss and boil in a boiling water bath for 1-2 minutes.

2. For samples rich in glucose and/or maltodextrins (e.g. processed foods and fermented foods)

Weigh50 mg sample into a 10ml tube. Add 2.5ml 80% v/v aqueous ethanol to wet the sample and incubate at 85°C for 5 min. Mix the tube on a vortex stirrer and add another 2.5 ml 80% v/v aqueous ethanol. Centrifuge the tube for 10 min at 3000 rpm and discard the supernatant. Resuspend the pellet in 5 ml 80% v/v aqueous ethanol and stir on a vortex mixer. Centrifuge the tube again for 10 min at 3000 rpm and discard the supernatant.Add 5ml distilled water to the sample and fasten down, then boil in a boiling water bath for 1-2 minutes.

VI. ASSAY PROCEDURE

1.Add following reagents intothe microcentrifuge tubes

Reagent*	Sample	Positive Control	Blank
Sample	5 ml	--	--
Positive Control	--	5 ml	--
Distilled water	--	--	5 ml
Enzyme I	25µl	25µl	25µl
Mix and incubate the tube in a boiling water bath for 6 min.			

2.Transfer 20 µl of boiled product to a new tube, then add following reagents.

Reagent*	Sample	Positive Control	Blank
Boiled product	20 µl	20 µl	20 µl
Enzyme II	25 µl	25 µl	25 µl
Mix and incubate the tube in a50°Cwater bath for 30 min. Centrifuge the tube at 3000 rpm for 10 min.			

3.Transfer 20µl supernatant tothe microplate, then add following reagents into it.

Reagent*	Sample**	Standard	Positive Control	Blank
Supernatant	20 µl	--	20 µl	20 µl
Standard	--	20 µl	--	--
Reaction Buffer	120 µl	120 µl	120 µl	120 µl
Enzyme III	10 µl	10 µl	10 µl	10 µl
Dye Reagent	50 µl	50 µl	50 µl	50 µl
Mix, put in the oven37°C for 15minutes, record absorbance measured at 550 nm.				

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.If reading exceeds

maximum value of standard curve, we recommend to appropriately diluent the boiling product from the Step 1 and proceed repeatedly according to Step 2. Thedilution factor is denoted as n.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

1. Calculate the sample concentration in ASSAY PROCEDURE according to the slope of the standard curve

$$C=\frac{(OD_{Sample}-OD_{Blank})-Intercept}{Slope}\times\frac{45}{20}\times 250\times\frac{162}{180}\times n(mg/ml)$$

Calculate the initial concentration according to sample preparation procedure.

2. According to one point of the standard OD and concentration

2.1. According to the weight of sample

$$C=\frac{(C_{Standard}\times V_{Standard})\times(OD_{Sample}-OD_{Blank})}{(OD_{Standard}-OD_{Blank})\times W\times V_{Sample}}\times\frac{45}{20}\times 250\times\frac{162}{180}\times n\ \ (mg/g)$$

Slope: the absorbance slope of standard curve

n: the dilution factor

$C_{Standard}$: the standard concentration, mg/ml

$V_{Standard}$: the volume of standard in assay procedure, µl

V_{Sample}: the volume of sample in assay procedure, µl

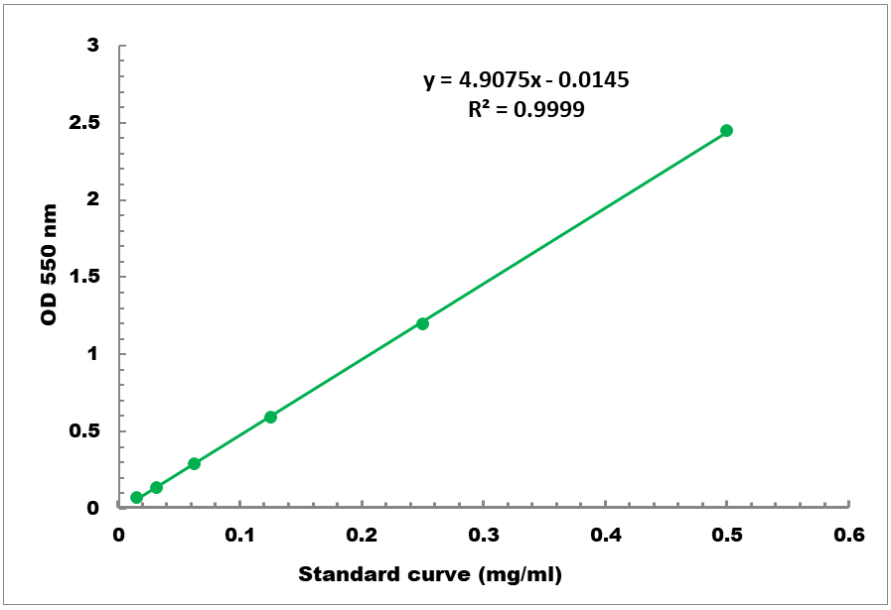
V_{Assay}: the volume of Assay Buffer, µl

W: the weight of sample, g

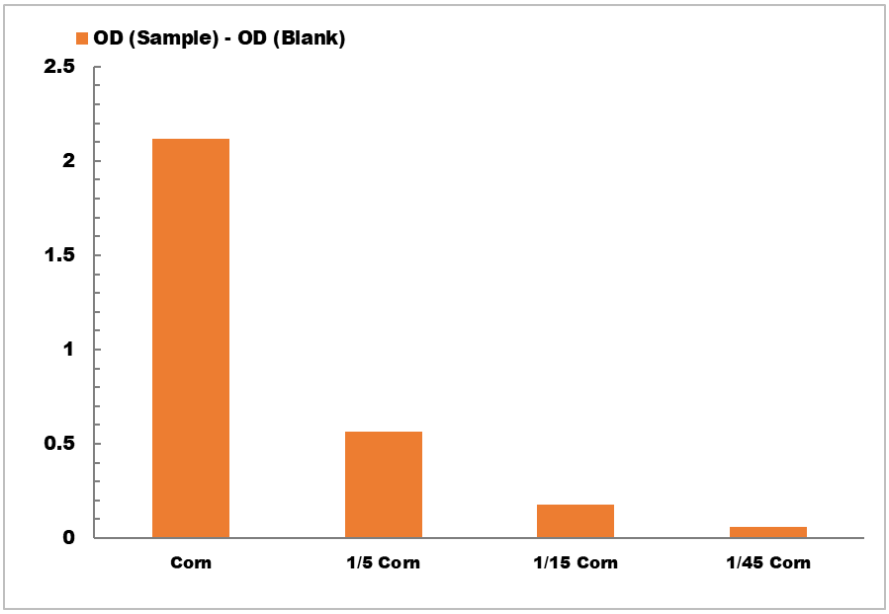
$\frac{162}{180}$:adjustment from free D-glucose to Starch

VIII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 0.01 mg/ml-0.5 mg/ml



Determination of starch in core