Starch Microplate Assay Kit

User Manual

Catalog # CAK1022

(Version 1.3C)

Detection and Quantification of Starch Content in Tissue extracts,

Cell lysate Samples.

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

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.

80% ethanol can be used to separate the soluble sugar and starch in the sample. And then decompose starch to glucose. The glucose content can be determined by anthrone colorimetry method, which can calculate starch content.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer	6 ml x 1	4 °C
Dye Reagent Diluent	15 ml x 1	4 °C
Dye Reagent	Powder x1	4 °C
Standard	Powder x1	4 °C
Plate Adhesive Strips	3 Strips	
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Note:

Standard: add 1 ml distilled water to dissolve before use, mix, heat in boiling water bath for 1 minute; the concentration will be 1 mg/ml.

Dye Reagent: add 15 ml Dye Reagent Diluent into the bottle, dissolve it absolutely before use, store at 4 °C.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 620 nm
2. Distilled water
3. Concentrated sulfuric acid
4. Pipettor, multi-channel pipettor
5. Pipette tips
6. Mortar
7. Ice
8. Centrifuge
9. Timer

IV. SAMPLE PREPARATION

1. For tissue samples

Weigh out 0.01 g tissue, homogenize with 1 ml Assay buffer, transfer it to the microcentrifuge tube, and put it in 80 °C water bath for 30 minutes, centrifuged at 3000g, room temperature for 5 minutes. Discard the supernatant.

Add 1 ml distilled water into the precipitate, then put it to the boiling water bath for 15 minutes (fasten down, in case moisture loss).

V. ASSAY PROCEDURE

Add following reagents into the microcentrifuge tubes:

Reagent	Sample	Standard	Blank
Sample	90 µl	--	--
Standard	--	90 µl	--
Reaction Buffer	60 µl	60 µl	--
Incubate at 95 °C for 15minutes, vortex 3 - 5 times; then centrifuged at 4000g for 10 minutes. Add following reagents into the microplate.			
Supernatant	50 µl	50 µl	--
Distilled water	--	--	50 µl
Dye Reagent	150 µl	150 µl	150 µl
Mix, put it in the oven, 90 °C for 15 minutes, record absorbance measured at 620 nm.			

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) 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.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

1. According to the weight of sample

$$\text{Starch (mg/g)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (W \times V_{Sample} / V_{Total})$$

$$= (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / W$$

$C_{Standard}$: the concentration of Standard, 1 mg/ml;

$C_{Protein}$: the protein concentration, mg/ml;

W: the weight of sample, g;

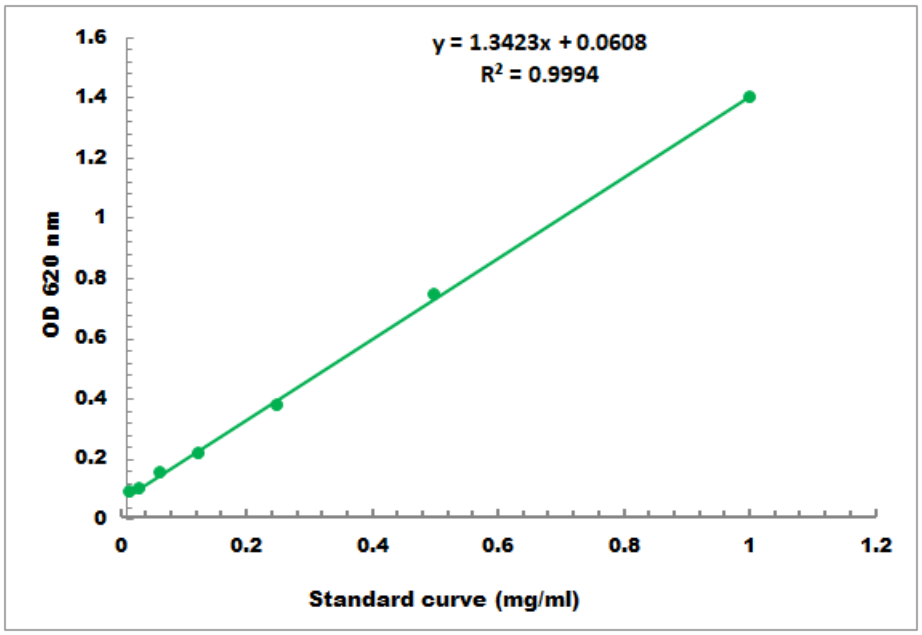
$V_{Standard}$: the volume of sample, 0.09 ml;

V_{Sample}: the volume of sample, 0.09 ml;

V_{Total}: the total volume of sample, 1 ml.

VII. TYPICAL DATA

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



Detection Range: 0.01 mg/ml - 1 mg/ml

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES