Amylopectin

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1179

(Version 1.3A)

Detection and Quantification of AmylopectinContent in Tissue extracts, Other biological fluidsSamples.

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

I. INTRODUCTION

Amylopectin is a water-soluble polysaccharide and highly branched polymer of α-glucose units found in plants. It is one of the two components of starch, the other being amylose.

AmylopectinColorimetric Microplate Assay Kit is a sensitive assay for determiningamylopectincontent in various samples.The purplish red is produced according to the action of amylopectin and iodine reagent. The measurement wavelength and reference wavelength of the amylose were 550nm and 735 nm.The absorbance difference between the two wavelengths is directly proportional to the content.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer A	10 ml x 1	4 °C
Reaction Buffer B	8 ml x 1	4 °C
Dye Reagent	1 mlx 1	4 °C, keep in dark
Standard	Powderx 1	4 °C
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III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 550nm and 735 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Centrifuge
6. Timer
7. Mortar

IV. REAGENT PREPARATION

Standard: Briefly centrifuge prior to opening. Dissolve in 1 ml Assay Buffer to generate 4 mg/ml of standard top solution, store at -20 °C for 1 month after reconstitution. Perform 2-fold serial dilutions of the top standard solution using Assay Buffer to make the standard curve. The concentration of standard curve could be 4/2/1/0.5/0.25/0.125 mg/ml.

V. SAMPLE PREPARATION

1.For Tissue samples

Weighout 0.01 g tissue, homogenize with 1 mlAssay Buffer, then transfer all the lysate to the microtube,centrifuged at 4000g for 10minutes; take the supernatant into a new centrifuge tube for detection.

VI. ASSAY PROCEDURE

Add following reagents intothe microplate:

Reagent*	Sample**	Standard	Blank
Sample	10 µl	--	--
Standard	--	10 µl	--
Distilled water	--	--	10 µl
Reaction Buffer A	100 µl	100 µl	100 µl
Reaction Buffer B	80 µl	80 µl	80 µl
Dye Reagent	10 µl	10 µl	10 µl
Mix, wait for 5 minutes, record absorbance measured at 550nm and 735nm.			

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.

**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. Calculatethe sample concentration in ASSAY PROCEDURE according to the slope of the standard curve

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

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

$$C=\frac{(C_{Standard}\times V_{Standard})\times(OD_{Sample}-OD_{Blank})}{(OD_{Standard}-OD_{Blank})\times W\times(V_{Sample}/V_{Assay})}(mg/g)$$

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

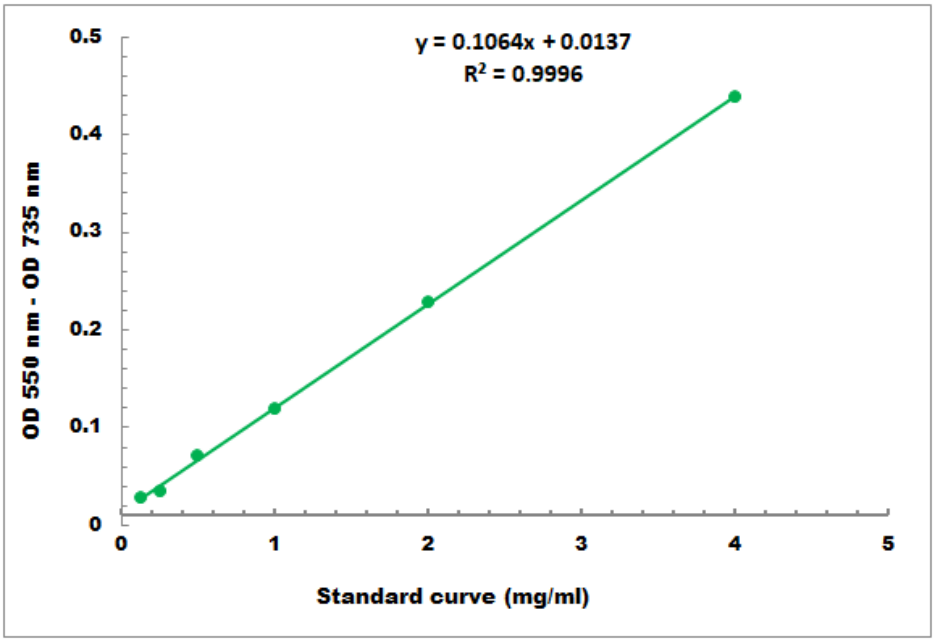
V_{Sample}: the volume of sample in assay procedure, µl

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

W: the weight of sample, g

VIII. TYPICAL DATA

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



Detection Range: 0.1 mg/ml - 4 mg/ml