Amylose Microplate Assay Kit

User Manual

Catalog # ASK1178

Detection and Quantification of Amylose content in Tissue extracts,

Other biological fluids Samples.

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

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I. INTRODUCTION

Amylose is a polysaccharide made of α-D-glucose units, bonded to each other through α(1→4) glycosidic bonds. It is one of the two components of starch, making up approximately 20-30%. Amylose is less soluble in water than the other component amylopectin. Because of its tightly packed helical structure, amylose is more resistant to digestion than other starch molecules and is therefore an important form of resistant starch.

Amylose Microplate Assay Kit is a sensitive assay for determining amylose content in various samples. The pure blue is produced according to the action of amylose and iodine reagent. The measurement wavelength and reference wavelength of the amylose were 630nm and 480 nm. The absorbance difference between the two wavelengths is directly proportional to the content.

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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 ml x 1	4 °C, keep in dark
Standard	Powder x 1	4 °C
Technical Manual	1 Manual	

Note:

Standard: add 1 ml Assay Buffer to dissolve before use, the concentration will be 4 mg/ml.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 630nm and 480nm
2. Distilled water
3. Pipettor
4. Pipette tips
5. Centrifuge
6. Timer
7. Mortar

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IV. SAMPLE PREPARATION

1. For Tissue samples

Weigh out 0.01 g tissue, homogenize with 1 ml Assay buffer, then transfer all the lysate to the microtube, centrifuged at 4000g for 10 minutes; take the supernatant into a new centrifuge tube for detection.

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V. ASSAY PROCEDURE

Add following reagents into the 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 630nm and 480nm.			

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VI. CALCULATION

1. According to the volume of sample

$$\text{Amylose (mg/ml)} = (C_{Standard} \times V_{Standard}) \times [(OD_{Sample630} - OD_{Sample480}) - OD_{Blank}] / [(OD_{Standard630} - OD_{Standard480}) - OD_{Blank}] / V_{Sample}$$

$$= 4 \times [(OD_{Sample630} - OD_{Sample480}) - OD_{Blank}] / [(OD_{Standard630} - OD_{Standard480}) - OD_{Blank}]$$

2. According to the weight of sample

$$\text{Amylose (mg/g)} = (C_{Standard} \times V_{Standard}) \times [(OD_{Sample630} - OD_{Sample480}) - OD_{Blank}] / [(OD_{Standard630} - OD_{Standard480}) - OD_{Blank}] / (V_{Sample} \times W / V_{Assay})$$

$$= 4 \times [(OD_{Sample630} - OD_{Sample480}) - OD_{Blank}] / [(OD_{Standard630} - OD_{Standard480}) - OD_{Blank}] / W$$

$C_{Standard}$: the standard concentration, 4 mg/ml;

W: the weight of sample, g;

V_{Assay}: the volume of Assay buffer, 1 ml

$V_{Standard}$: the volume of standard, 10 µl;

V_{Sample}: the volume of sample, 10 µl.

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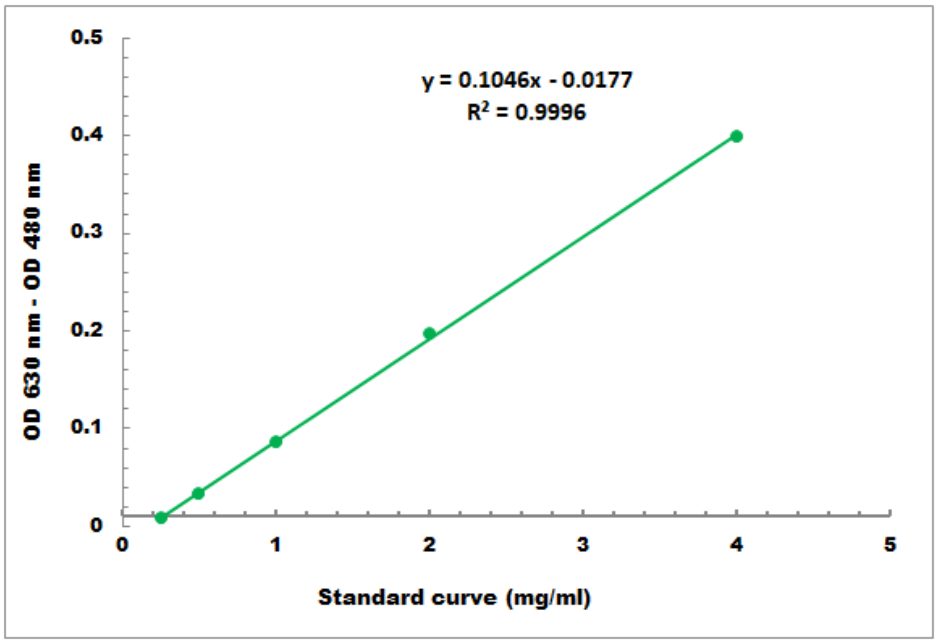
VII. TYPICAL DATA

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



Detection Range: 0.4 mg/ml - 4 mg/ml

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