

Amylose Microplate Assay Kit

Cat #: orb545634 (manual)

Detection and Quantification of Amylose content in Tissue extracts, Other biological fluids Samples.

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

INTRODUCTION

Amylose is a polysaccharide made of α -D-glucose units, bonded to each other through α (1 \rightarrow 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.

KIT COMPONENTS

| Component | Volume | Storage |
|--------------------|------------|--------------------|
| 96-Well Microplate | 1 plate | |
| Assay Buffer I | 30 ml x 4 | 4 °C |
| Assay Buffer II | 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 II to dissolve before use, the concentration will be 8 mg/ml.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 630nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Centrifuge
6. Timer
7. Mortar

SAMPLE PREPARATION

1. For tissue samples

Weigh out 0.01 g tissue, homogenize with 1 ml Assay Buffer I, then transfer all the lysate to the microtube, centrifuged at 4000g for 10 minutes, discards the supernatant; then add 1 ml Assay Buffer II, warm at 80 °C for 10 mins, centrifuged at 4000g for 10 minutes, take the supernatant into a new centrifuge tube for detection.

ASSAY PROCEDURE

Add following reagents into the microplate:

| Reagent | Sample | Standard | Blank |
|-------------------|-------------|-------------|-------------|
| Sample | 10 μ l | -- | -- |
| Standard | -- | 10 μ l | -- |
| Distilled water | -- | -- | 10 μ l |
| Reaction Buffer A | 100 μ l | 100 μ l | 100 μ l |
| Reaction Buffer B | 80 μ l | 80 μ l | 80 μ l |
| Dye Reagent | 10 μ l | 10 μ l | 10 μ l |

Mix, wait for 5 minutes, record absorbance measured at 630nm.

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.

CALCULATION

1. According to the volume of sample

$$\begin{aligned}\text{Amylose (mg/ml)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / V_{\text{Sample}} \\ &= 8 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}})\end{aligned}$$

2. According to the weight of sample

$$\begin{aligned}\text{Amylose (mg/g)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / (V_{\text{Sample}} \times W / V_{\text{Assay}}) \\ &= 8 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / W\end{aligned}$$

C_{Standard} : the standard concentration, 8 mg/ml;

W : the weight of sample, g;

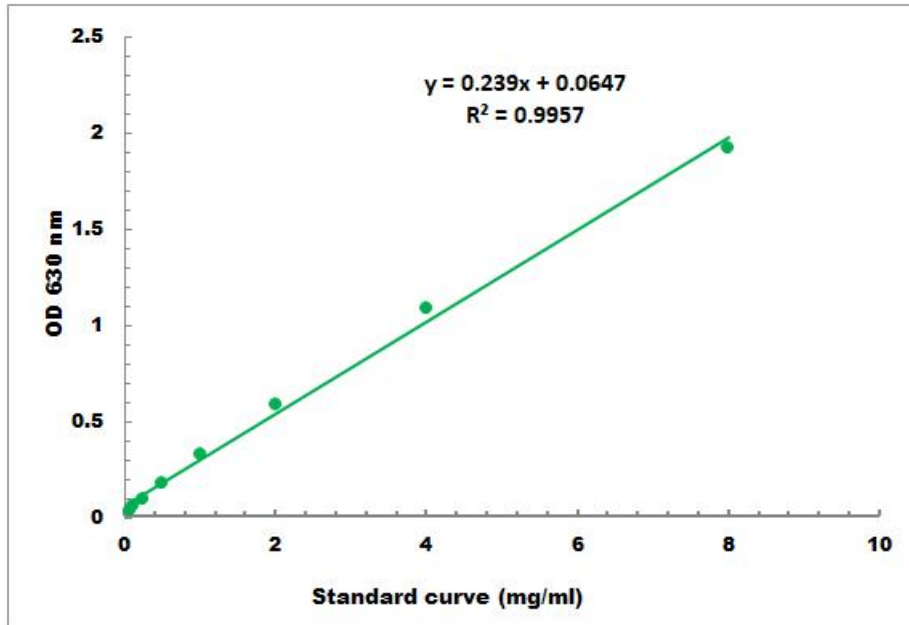
V_{Assay} : the volume of Assay buffer II, 1 ml

V_{Standard} : the volume of standard, 10 μ l;

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

TYPICAL DATA

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



Detection Range: 0.8 mg/ml - 8 mg/ml