



Beta-Amylase Microplate Assay Kit

User Manual

Catalog # ASK1024

Detection and Quantification of Beta-Amylase Activity in Tissue extracts, Cell lysate Samples.

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

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**I. INTRODUCTION**

Amylase belongs to the family of glycoside hydrolase enzymes that break down starch into glucose molecules by acting on α -1,4-glycosidic bonds. β -Amylase plays a central role in the complete degradation of starch to metabolisable or fermentable sugars during the germination or malting of cereal grains. It also finds considerable application, together with starch debranching enzymes, in the production of high maltose syrups. β -Amylase is usually measured using non-specific reducing sugar assays with starch as substrate.

Amylolytic enzyme hydrolyzes the starch to generate reducing sugar. The reducing sugar reduces the 3,5-dinitrosalicylic acid to generate red-brown substance. The color intensity, measured at 540 nm, is proportionate to the enzyme activity in the sample.

**II. KIT COMPONENTS**

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer	4 ml x 1	4 °C
Substrate	Powder x 1	4 °C
Stop Solution	4 ml x 1	4 °C
Dye Reagent	20 ml x 1	4 °C
Standard	Powder x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

Note:

Substrate: add 8 ml distilled water to dissolve before use.

Standard: add 1 ml distilled water to dissolve before use; then add 0.1 ml into 0.9 ml distilled water, the concentration will be 2 mmol/L.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 540 nm
2. Distilled water
3. Pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Convection oven



IV. SAMPLE PREPARATION

1. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay Buffer on ice, transfer it to the microcentrifuge tube, extract for 15 minutes at 4 °C, vortex 3 - 5 times, centrifuged at 3000g, 4 °C for 10 minutes. Take the supernatant into a new tube.

**V. ASSAY PROCEDURE**

Add following reagents in the microplate:

Reagent	Sample	Control	Standard	Blank
Sample	--	20 μ l	--	--
Put it in the oven, 70 °C for 15 minutes. Then put it on ice.				
Reaction Buffer	20 μ l	20 μ l	--	--
Sample	20 μ l	--	--	--
Substrate	40 μ l	40 μ l	--	--
Mix, put it in the oven, 40 °C for 5 minutes.				
Stop Solution	20 μ l	20 μ l	--	--
Standard	--	--	100 μ l	
Distilled water	--	--	--	100 μ l
Dye Reagent	100 μ l	100 μ l	100 μ l	100 μ l
Mix, put it into the convection oven, 90 °C for 10 minutes, record absorbance measured at 540nm.				

**VI. CALCULATION**

Unit Definition: One unit of β -Amylase activity is defined as the enzyme generates 1 μ mol of reducing sugar per minute.

1. According to the protein concentration of sample

$$\begin{aligned}\beta\text{-Amylase (U/mg)} &= C_{\text{Standard}} \times V_{\text{Standard}} \times (OD_{\text{Sample}} - OD_{\text{Control}}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) / \\ &\quad V_{\text{Sample}} / C_{\text{Protein}} / T \\ &= 2 \times (OD_{\text{Sample}} - OD_{\text{Control}}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) / C_{\text{Protein}}\end{aligned}$$

2. According to the weight of sample

$$\begin{aligned}\beta\text{-Amylase (U/g)} &= C_{\text{Standard}} \times V_{\text{Standard}} \times (OD_{\text{Sample}} - OD_{\text{Control}}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) / \\ &\quad (V_{\text{Sample}} \times W / V_{\text{Assay}}) / T \\ &= 2 \times (OD_{\text{Sample}} - OD_{\text{Control}}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) / W\end{aligned}$$

C_{Standard} : the standard concentration, 2 mmol/L = 2 μ mol/ml;

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

W: the weight of sample, g;

V_{Standard} : the volume of standard, 0.1 ml;

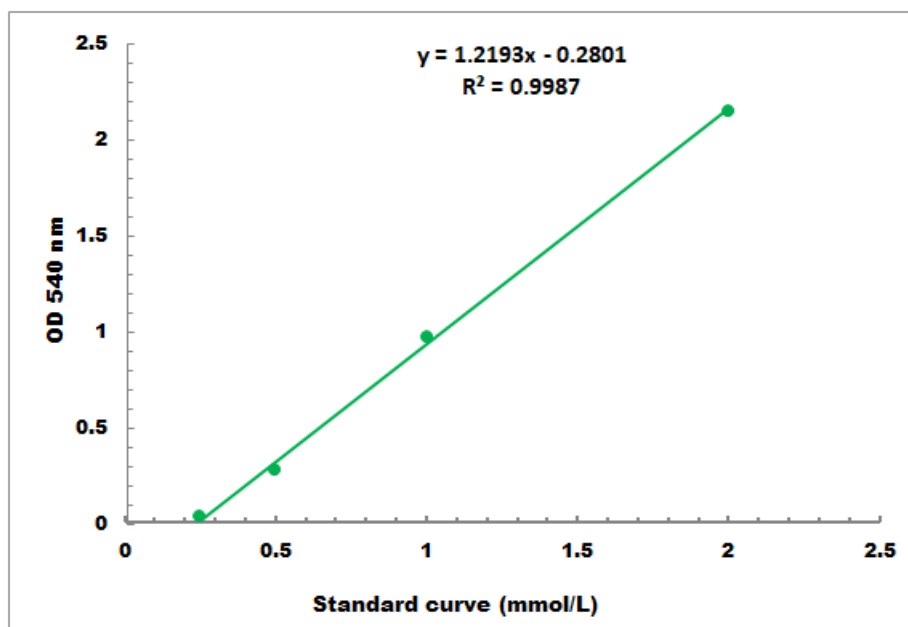
V_{Sample} : the volume of sample, 0.02 ml;

V_{Assay} : the volume of Assay Buffer, 1 ml;

T: the reaction time, 5 minutes.

VII. TYPICAL DATA

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



Detection Range: 0.2 mmol/L - 2 mmol/L