Beta-Amylase Microplate Assay Kit

Catalog # AS0024

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

This instruction must be read in its entirety before using this product.

For research use only, Not for use in diagnostic 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 mlx 4	4 °C
Reaction Buffer	4 mlx 1	4 °C
Substrate	Powderx 1	4 °C
Stop Solution	4 mlx 1	4 °C
Dye Reagent	20 ml x 1	4 °C
Standard	Powderx 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

Note:

Substrate:add 8 mldistilled water to dissolve before use,mix, heat in boiling water bath for 1 minute.

Standard:add 1 mldistilled water to dissolve before use; then add 0.1 ml into 0.9 mldistilled 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

Weighout 0.1 g tissue, homogenize with 1 mlAssay Buffer on ice, transfer it to the microcentrifuge tube,extract for 15minutes 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 µl	--	--
Put it in the oven, 70 °C for 15minutes. Then put it on ice.				
Reaction Buffer	20 µl	20 µl	--	--
Sample	20 µl	--	--	--
Substrate	40 µl	40 µl	--	--
Mix, put it in the oven, 40°C for 5 minutes.				
Stop Solution	20 µl	20 µl	--	--
Standard	--	--	100 µl	
Distilled water	--	--	--	100 µl
Dye Reagent	100 µl	100 µl	100 µl	100 µl
Mix, put it into the convection oven,90 °Cfor 10 minutes, record absorbance measured at 540nm.				

VI. CALCULATION

Unit Definition: One unit ofβ-Amylase activity is defined as the enzyme generates 1 μmolof reducing sugar per minute.

1. According to the protein concentration of sample

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

$$= 2 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

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

$$= 2 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / W$$

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

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

W: the weight of sample, g;

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

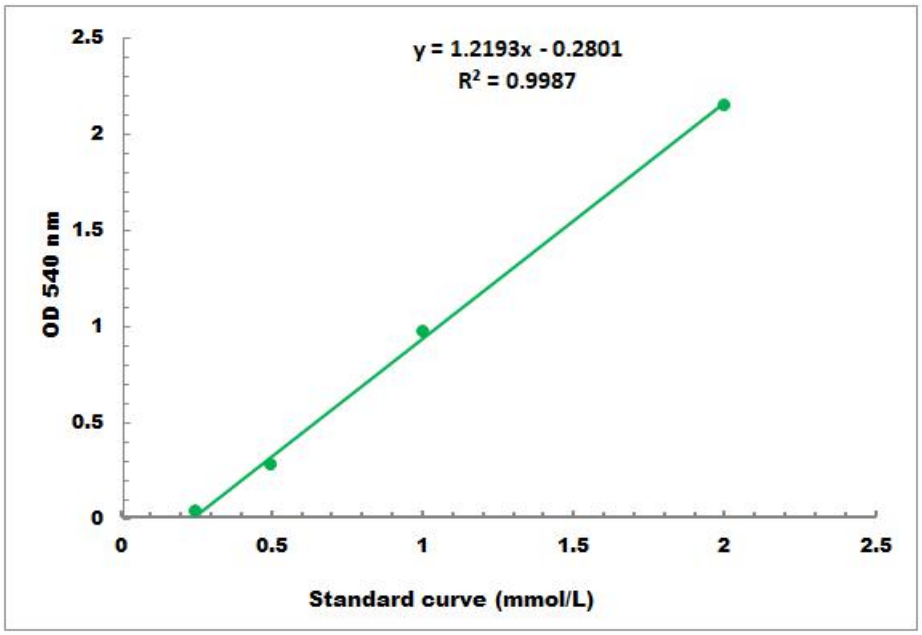
V_{Sample}: the volume of sample, 0.02 ml;

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

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.sabbiotech.cn or contact us at techcn@signalwayantibody.com

IX. NOTES