Beta-Amylase Microplate Assay Kit

User Manual

Catalog # CAK1024

(Version 1.3D)

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

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

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	5 ml x 1	4 °C
Substrate	Powder x 1	4 °C
Dye Reagent	10 ml x 1	4 °C
Standard	Powder x 1	4 °C
Positive Control	Powder x 1	-20 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

Note:

Substrate: add 4 ml distilled water to dissolve before use, mix, heat in boiling water bath for 1 minute.

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.

Positive Control: add 0.2 ml distilled water to dissolve before use, mix.

III. MATERIALS REQUIRED BUT NOT PROVIDED

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

IV. SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay buffer for 5×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times); centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay buffer on ice, centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For liquid samples

Detect directly.

V. ASSAY PROCEDURE

Add following reagents in the microplate:

Reagent	Sample	Control	Standard	Blank	Positive Control
Reaction Buffer	50 µl	50 µl	--	--	50 µl
Sample	10 µl	--	--	--	--
Distilled water	--	10 µl	--	--	--
Positive Control	--	--	--	--	10 µl
Substrate	40 µl	40 µl	--	--	40 µl
Mix, put it in the oven, 40 °C for 10 minutes.					
Standard	--	--	100 µl	--	--
Distilled water	--	--	--	100 µl	--
Dye Reagent	100 µl	100 µl	100 µl	100 µl	100 µl
Mix, put it into the convection oven, 90 °C for 10 minutes, record absorbance measured at 540nm.					

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more sample into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

Unit Definition: One unit of β-Amylase activity is defined as the enzyme generates 1 μmol of 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;

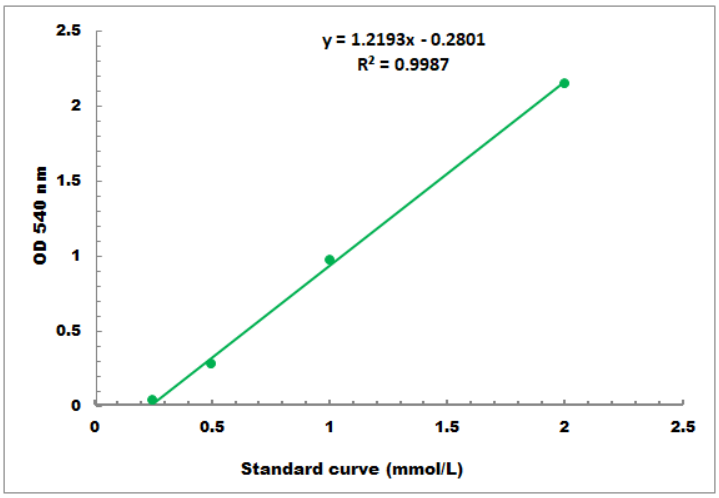
V_{Sample}: the volume of sample, 0.01 ml;

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

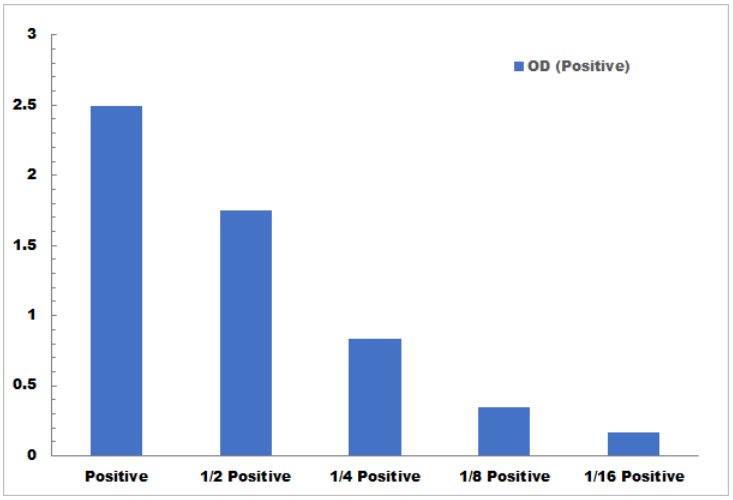
T: the reaction time, 10 minutes.

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



Positive Control reaction in 96-well plate assay with decreasing the concentration

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES