Alpha-Amylase Microplate Assay Kit

Cat #: orb390729 (manual)

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

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

Biorbyt Ltd
7 Signet Court, Swann Road, Cambridge, CB5 8LA. United Kingdom
Email: info@biorbyt.com | Phone: +44 (0)1223 859-353 | Fax: +44 (0)1223 280-240

Biorbyt LLC
68 TW Alexander Drive, Durham, NC 27709. United States
Email: info@biorbyt.com | Phone: +1 (415) 906-5211 | Fax: +1 (415) 651-8558

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. The α-amylases cleave at random locations on the starch chain, ultimately yielding maltotriose and maltose, glucose and "limit dextrin" from amylose and amylopectin. In mammals, α-amylase is a major digestive enzyme. Increased enzyme levels in humans are associated with salivary trauma, mumps due to inflammation of the salivary glands, pancreatitis and renal failure.

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.

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 0.1 ml distilled water to dissolve before use, mix.

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

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.

ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent	Sample	Control	Standard	Blank	Positive Control
Sample	10 µl	--	--	--	--
Distilled water	--	10 µl	--	--	--
Positive Control	--	--	--	--	10 µl
Put it in the oven, 70 °C for 15 minutes.					
Reaction Buffer	50 µl	50 µl	--	--	50 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
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.

CALCULATION

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

1. According to the protein concentration of sample

$$\alpha\text{-Amylase (U/mg)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / V_{Sample} / C_{Protein} / T$$
$$= 2 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

$$\alpha\text{-Amylase (U/g)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times W / V_{Assay}) / T$$
$$= 2 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / W$$

$C_{Standard}$: 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;

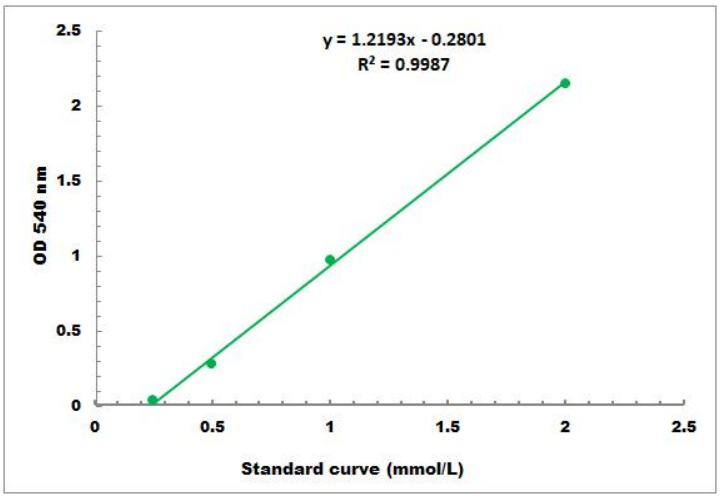
V_{Sample}: the volume of sample, 0.01 ml;

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

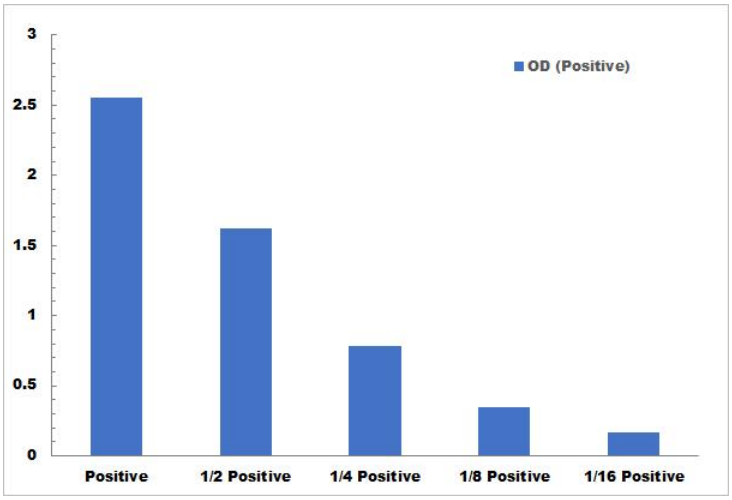
T: the reaction time, 10 minutes.

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

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