



# Alpha-Amylase Microplate Assay Kit

**Catalog # AS0023**

Detection and Quantification of Alpha-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  $\alpha$ -1,4-glycosidic bonds. The  $\alpha$ -amylases cleave at random locations on the starch chain, ultimately yielding maltotriose and maltose, glucose and "limit dextrin" from amylose and amylopectin. In mammals,  $\alpha$ -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.

## 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, 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.

## 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, transfer it to the microcentrifuge tube, extract for 15 minutes, vortex 3 - 5 times, centrifuge at 3000g, room temperature for 10 minutes. Take the supernatant into a new tube.

## V. ASSAY PROCEDURE

Add following reagents into the microplate:

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

## VI. CALCULATION

**Unit Definition:** One unit of  $\alpha$ -Amylase activity is defined as the enzyme generates 1  $\mu$ mol of reducing sugar per minute.

1. According to the protein concentration of sample

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

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

2. According to the weight of sample

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

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

$C_{\text{Standard}}$ : the standard concentration, 2 mmol/L = 2  $\mu$ mol/ml;

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

W: the weight of sample, g;

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

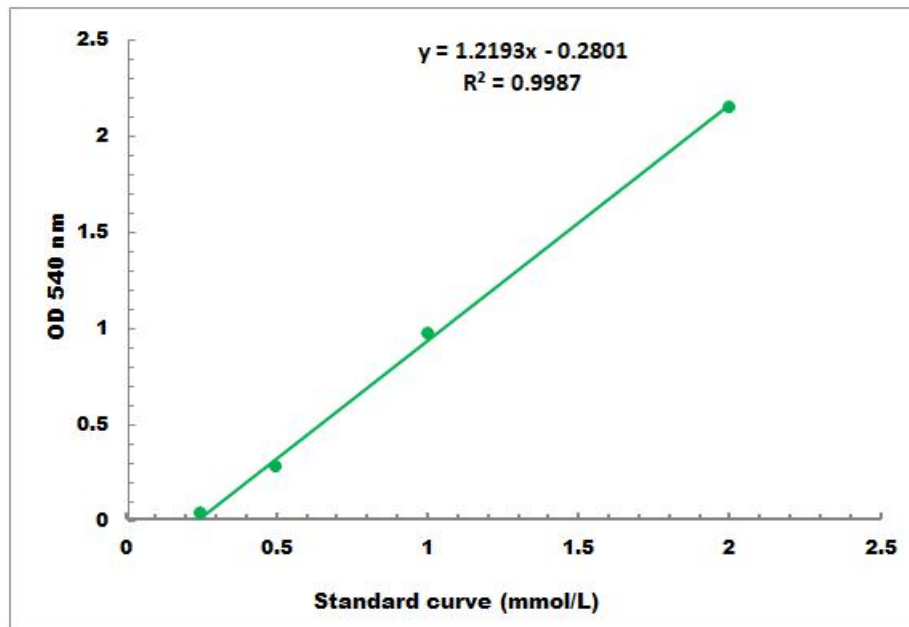
$V_{\text{Sample}}$ : the volume of sample, 0.02 ml;

$V_{\text{Assay}}$ : 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](http://www.sabbiotech.cn) or contact us at [techcn@signalwayantibody.com](mailto:techcn@signalwayantibody.com)

## IX. NOTES