(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS!)

β-Amylase (AMS) Activity Assay Kit

Catalog No.: BC00138

Size: 50T

Please read the instructions carefully before use. If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

✉ Email (Sale) order@enkilife.com

✉ Email (Techsupport) techsupport@enkilife.com

☎ Tel: 0086-27-87002838

Website: www.enkilife.com

Shelf life: Please refer to the label on the outer package.

Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Basic Information

Product Name	β-Amylase (AMS) Activity Assay Kit
Detection Method	Colorimetric
Sample Type	Tissue, serum, plasma
Assay Type	Quantitative
Detection Instrument	Visible spectrophotometer or microplate reader (540 nm)

Product Introduction

Amylases are responsible for hydrolyzing starch, and mainly include α-amylase and β-amylase. β-amylase can randomly act on the α-1,4-glycosidic bonds in starch to produce reducing sugars such as glucose, maltose, maltotriose, and dextrin.

Principle

Reducing sugars reduce 3,5-dinitrosalicylic acid to produce a brownish-red substance. α-Starch is intolerant of acid, and β-amylase is intolerant of heat. Based on these characteristics, by passivating one of them, the activity of the other amylase can be measured.

Components

Components	Name	Size	Storage
Reagent 1	Substrate liquid	30 mL	RT
Reagent 2	Colorimetric	60 mL	Store at 4℃
Reagent 3	Sugar standard	2 mL (concentration 1 mg/mL)	Store at 4℃
Consumables 1	Microplate (96 wells)	1 plate	RT
Consumables 2	Plate Sealer	2 pieces	RT

Note: If only standard tubes are being measured without creating a standard curve, a 0.25 mg/mL standard working solution can be prepared by adding distilled water at a ratio of 1:3. Prepare only the amount needed.

Storage

This kit is stored under the specified conditions upon receipt and has a shelf life of 3 months.

Preparation before the experiment

Sample processing (crude enzyme extraction)

1. **Preparation:** Weigh 0.1–0.2 g of sample (approximately 0.1 g is recommended), add 1 mL of distilled water, and grind into a homogenate. Pour the homogenate into a centrifuge tube and allow the extract to stand at room temperature for 15 min (shaking once every 5 min) to ensure complete extraction. Centrifuge at 3000 g for 10 min at room temperature, take 0.5 mL of the supernatant, add 2 mL of distilled water, and mix well. This is the amylase stock solution, used for the determination of α-amylase activity. Take 0.5 mL of the above amylase stock solution, add 4.5 mL of distilled water (10-fold dilution), and mix well. This is the amylase dilution solution, used for the determination of total (α+β) amylase activity.
2. **For liquid samples such as serum (plasma)**: ① Directly detect α-amylase activity ② Take 0.5 mL of sample, add 4.5 mL of distilled water, mix well, and this is the amylase dilution solution, which is used for the determination of total (α+β)amylase activity.

Operation process

Reagent Name	α - Amylase		Total amylase		Blank tube	Standard tube
	Test tube (A1)	Control tube (A2)	Test tube (A3)	Control tube (A4)		
Amylase stock solution (μL)	250	250	--	--	--	--
	70 ℃ water bath for 15 minutes		--	--	--	--

Amylase dilution (μL)	--	--	250	250	--	--
Distilled water (μL)	--	--	--	--	500	500
0.25 mg/mL standard solution (μL)	--	--	--	--	--	250
Reagent 1 (μL)	250	--	250	--	--	--
40 ℃ constant temperature water bath for 15 minutes					--	--
Reagent 2 (μL)	500	500	500	500	500	500
Reagent 1 (μL)	--	250	--	250	--	--
Mix well, incubate in a 95 ℃ water bath for 5 min, and measure the absorbance of each tube using a 1 cm path length cuvette at a wavelength of 540 nm.						

Result calculation

1. Tissue amylase activity:

(1) Calculated based on sample quality

Unit definition: One enzyme activity unit is defined as the amount of reducing sugar produced per g of tissue per minute by catalysis of 1 mg of reducing sugar.

$$\alpha-\text{Amylase activity (U/g tissue)} = \frac{A1-A2}{A_{blank}-A_{standard}} \times C_{standard} \times V_{standard} \div (W \div V_{extract} \div 5 \times V_{sample}) \div T$$

$$\text{Total Amylase activity (U/g tissue)} = \frac{A3-A4}{A_{blank}-A_{standard}} \times C_{standard} \times V_{standard} \div (W \div V_{extract} \div 5 \times V_{sample}) \div T \times 10$$

$$\beta-\text{Amylase activity (U/g tissue)} = \text{Total Amylase activity} - (\alpha-\text{Amylase activity})$$

(2) Calculated based on protein content

Unit definition: One enzyme activity unit is defined as the amount of reducing sugar produced by the catalysis of 1 mg of tissue protein per minute.

$$\alpha-\text{Amylase activity (U/mgprot)} = \frac{A1-A2}{A_{blank}-A_{standard}} \times C_{standard} \times V_{standard} \div (V_{sample} \times Cpr) \div T$$

$$\text{Total Amylase activity (U/mgprot)} = \frac{A3-A4}{A_{blank}-A_{standard}} \times C_{standard} \times V_{standard} \div (V_{sample} \times Cpr) \div T \times 10$$

$$\beta-\text{Amylase activity (U/mgprot)} = \text{Total Amylase activity} - (\alpha-\text{Amylase activity})$$

2. Calculation of amylase activity in liquid samples such as serum (plasma):

Unit definition: One enzyme activity unit is defined as the amount of reducing sugar produced per milliliter of sample per minute by catalysis of 1 mg.

$$\alpha-\text{Amylase activity (U/mLt)} = \frac{A1-A2}{A_{blank}-A_{standard}} \times C_{standard} \times V_{standard} \div V_{sample} \div T$$

$$\text{Total Amylase activity (U/ml)} = \frac{A3-A4}{A_{blank}-A_{standard}} \times C_{standard} \times V_{standard} \div V_{sample} \div T \times 10$$

$$\beta-\text{Amylase activity (U/mL)} = \text{Total Amylase activity} - (\alpha-\text{Amylase activity})$$

Annotation

$C_{standard}$: standard solution concentration, 0.25 mg/mL;

$V_{standard}$: Standard solution added, 0.25 mL;

V_{sample} : The amount of sample added during the reaction, 0.25 mL;

$V_{extract}$: Extraction volume, 1 mL ;

W: Sample mass, g;

T: Reaction time, 5 min;

Cpr: Amylase stock solution protein concentration, mgprot/mL (prot refers to protein), protein assay kit (BCA 00006);

5: The dilution factor when preparing amylase stock solution;

10: Dilution factor before total amylase test (if the total amylase activity of the sample is high, this dilution factor can be increased appropriately, and vice versa)

Precautions

1. When using microplate reader for this method, the reaction cannot be performed directly in the well plate. Instead, after the reaction is complete in the tube, 0.2 mL of the reaction solution should be transferred to a 96-well plate and the reading should be taken at 540 nm.
2. The reagent may precipitate solids during storage and must be dissolved at 70°C before use.
3. Different samples have different amylase activities (just like commercially available flour, which varies greatly between different times and varieties), so it is essential to conduct preliminary experiments to ensure the experimental results are satisfactory.
4. This product is for research purposes only and must not be used for clinical diagnosis. Do not take it.