ELK Biotechnology
For research use only

BC090β- amylase (AMS) test kit

CAT/NO.: BC090 Pack: 50T/24S

1. Assay significance

Amylase is responsible for the hydrolysis of starch, primarily α-amylase and β-amylase(EC 3.2.1.2), which acts randomly on 1,4-glycosidic bonds in starch to produce reducing sugars such as glucose, maltose, maltotriose, and dextrin.

2. Assay principle

Reducing Sugar Reduces 3,5-dinitrosalicylic acid to a brownish red substance. The α-amylase is not acid-resistant.and β-amylase is not heat-resistant. According to the above characteristics, inactivation of one of them, you can measure the activity of another amylase.

3. Additional Materials Required

·Visible photometer
·Water bath tank and Centrifuges
·Adjustable pipette (5-1000μl) and Tips
·1ml glass cuvette
·Mortar and Distilled water

4. Reagents composition（50T/24S）

Reagent 1:50ml×1 bottle,store at room temperature, If there is yellow crystal precipitation, it should be heated at 90°C and dissolved before use.
Reagent 2:40ml×1 bottle,store at 4℃.If there is precipitation, it should be heated at 70°C and dissolved before being used.

5. Operation procedure

1. Extraction of crude enzyme solution

Tissue: Weigh 0.1-0.2g ample (recommended weigh about 0.1 g) and crush in mortar, add 1ml distilled water, homogenize thoroughly and transfer to EP tube, extract at room temperature for 15min, oscillate once every 5 minutes to make it fully extracted,3,000g, centrifuged at room temperature for 10min, take the supernatant and add distilled water to 10ml, mix well to prepare **Amylase stock solution.** Absorbe the above amylase stock solution 1ml,add 4ml distilled water,mix well to prepare **Amylase diluent.** Used for the

determination of total activity of (α＋β) amylase.

Serum, plasma, and other fluids: (1) Direct detect α-amylase (2) Absorbe 1ml sample,add 4ml distilled water,mix well to prepare **Amylase diluent.**Used for the determination of total activity of (α＋β) amylase.

2. Operation procedure

①. **Preheat the photometer for more than 30 minutes, adjust the wavelength to 540nm and set zero with distilled water.**

②. Preheat the reagent 1 and reagent 2 at 40℃ for 10 min.

③. **Operation table**

Reagents	α-Amylase		Total Amylase	
	Contrast （A1）	Assay (A2)	Contrast (A3)	Assay (A4)
Amylase stock solution（μl）	250	250		
Incubate in water bath at 70°C for 15 min and cooled naturally				
Amylase diluent（μl）			250	250
Distilled water（μl）	250		250	
Reagent 2（μl）		250		250
Incubated at 40°C water bath tank for 5 min				
Reagent 1（μl）	500	500	500	500
Mix well and incubate 5min at 95℃,natural cooling to room temperature, Wavelength 540nm, 1ml cuvette,adjust zero with distilled water,determine absorbance value of each tube A_1. A_2. A_3. A_4				

6. Calculate

1. Determination of regression curve under standard conditions

y=3.7215x -0.1778； x is standard solution concentration (mg/ml), y is the absorbance value.

2. α-Amylase activity calculate

（1）Calculated by sample fresh weight

Unit definition:In this system, 1 mg reducing sugar catalyzed by per gram of tissue per minute is defined as 1 unit of enzyme activity.

$$\text{α-Amylase Activity（U/g Tissue）}=[(A_2\text{-}A_1+0.1778)\div 3.7215\times V_{\text{Total Volume}}]\div(V_{\text{Sample}}\div V_{\text{Total Sample}}\times W)\div T=1.075\times(A_2-A_1+0.1778)\div W$$

（2）Calculate by protein concentration

Unit definition:In this system, 1 mg reducing sugar catalyzed by per mg protein per minute is defined as 1 unit of enzyme activity.

$$\text{α-Amylase Activity（U/mgprot）}=[(A_2-A_1+0.1778)\div 3.7215\times V_{\text{Total Volume}}]\div(V_{\text{Sample}}\times C_{pr})\div T=0.1075\times(A_2-A_1+0.1778)\div C_{pr}$$

（3）Calculate by sample volume

Unit definition:In this system, 1 mg reducing sugar catalyzed by per ml serum,blood plasma per minute is defined as 1 unit of enzyme activity.

$$\alpha\text{-Amylase Activity (U/ml)} = [(A_2-A_1+0.1778)\div 3.7215\times V_{\text{Total Volume}}]\div V_{\text{Sample}}\div T=0.1075\times(A_2-A_1+0.1778)$$

3. Total Amylase activity calculate

（1）Calculated by sample fresh weight

Unit definition:In this system, 1 mg reducing sugar catalyzed by per gram of tissue per minute is defined as 1 unit of enzyme activity.

$$\text{Total Amylase Activity (U/g Tissue)} = 5\times[(A_4-A_3+0.1778)\div 3.7215\times V_{\text{Total Volume}}]\div(V_{\text{Sample}}\div V_{\text{Total Sample}}\times W)\div T=5.375\times(A_4-A_3+0.1778)\div W$$

（2）Calculate by protein concentration

Unit definition:In this system, 1 mg reducing sugar catalyzed by per mg protein per minute is defined as 1 unit of enzyme activity.

$$\text{Total Amylase Activity (U/mgprot)} = 5\times[(A_4-A_3+0.1778)\div 3.7215\times V_{\text{Total Volume}}]\div(V_{\text{Sample}}\div Cpr)\div T=0.5375\times(A_4-A_3+0.1778)\div Cpr$$

（3）Calculate by sample volume

Unit definition:In this system, 1 mg reducing sugar catalyzed by per ml serum,blood plasma per minute is defined as 1 unit of enzyme activity.

$$\text{Total Amylase Activity (U/ml)} = 5\times[(A_4-A_3+0.1778)\div 3.7215\times V_{\text{Total Volume}}]\div V_{\text{Sample}}\div T=0.5375\times(A_4-A_3+0.1778)$$

4. β-Amylase activity calculate

（1）Calculated by sample fresh weight

Unit definition:In this system, 1 mg reducing sugar catalyzed by per gram of tissue per minute is defined as 1 unit of enzyme activity.

$$\beta\text{-Amylase Activity (U/g Tissue)} = \text{Total Amylase Activity}-\alpha\text{-Amylase Activity} = [5.375\times(A_4-A_3+0.1778)-1.075\times(A_2-A_1+0.1778)]\div W$$

（2）Calculate by protein concentration

Unit definition:In this system, 1 mg reducing sugar catalyzed by per mg protein per minute is defined as 1 unit of enzyme activity.

$$\beta\text{-Amylase Activity (U/mgprot)} = \textit{Total Amylase Activity}-\textit{a-Amylase Activity} = [0.5375\times(A_4-A_3+0.1778)-0.1075\times(A_2-A_1+0.1778)]\div C_{pr}$$

（3）Calculate by sample volume

Unit definition:In this system, 1 mg reducing sugar catalyzed by per ml serum,blood plasma per minute is defined as 1 unit of enzyme activity.

$$\underset{(U/ml)}{\beta\text{-Amylase Activity}} = \text{Total Amylase Activity} - \alpha\text{-Amylase Activity} = 0.5375 \times (A_4 - A_3 + 0.1778) - 0.1075 \times (A_2 - A_1 + 0.1778)$$

5：Dilution times of total amylase

$V_{Total\ Volume}$: Total volume of reaction system, 0.5ml

V_{Sample}： Sample volume added to the reaction system,0.25 ml

$V_{Total\ Sasmple}$： Total volume of sample extract solution,10 ml

Cpr： sample protein concentration,mg/ml

W： Sample quality,g

T： Reaction Time,5min