CheKine™ Micro β-Amylase Activity Assay Kit

Cat #: KTB1380 Size: 48 T/48 S 96 T/96 S

	Micro β-Amylase Activity Assay Kit		
REF	**Cat #:** KTB1380	LOT	**Lot #:** Refer to product label
	Detection range: 0.0156-1 mg/mL		**Sensitivity:** 0.0078 mg/mL
	Applicable samples: Serum, Plasma, Saliva, Animal and Plant Tissues		
	Storage: Stable for 12 months at 4℃, protected from light		

Note: The detection range and sensitivity here refer to standard, which need to be converted to β-Amylase activity based on sample conditions.

Assay Principle

Amylase is responsible for hydrolyzing starch, includes α-amylase (α-AL) and β-amylase. β-Amylase (β-AL, EC 3.2.1.2) cuts α-1,4 glycosidic bonds from the non-reducing end of starch to generate reducing sugars such as glucose, maltose, maltotriose, and dextrin. CheKine™ Micro β-Amylase Activity Assay Kit provides a convenient tool for sensitive detection of β-AL activity. The principle is that amylase catalyzes the hydrolysis of starch to form reducing sugars, and 3,5-dinitrosalicylic acid is reduced by reducing sugar to produce a brown-red substance with an absorption peak at 540 nm; the amylase activity is calculated by measuring the rate of increase in absorbance at 540 nm. α-AL is heat-resistant, but β-amylase can be inactivated at 70℃ for 15 min. Therefore, only α-AL can catalyze starch hydrolysis after the crude enzyme solution is passivated at 70℃ for 15 min. The total amylase activity is measured with the crude enzyme solution that is not passivated, and the β-amylase activity can be obtained by subtracting the α-amylase form total amylase activity.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
DNS Reagent	37.5 mL	75 mL	4℃, protected from light
Substrate	Powder×1 vial	Powder×1 vial	4℃
Standard	Powder×1 vial	Powder×1 vial	4℃

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 540 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips

- Refrigerated centrifuge, water bath, ice maker, incubator
- Deionized water
- Homogenizer (for tissue samples)

Reagent Preparation

DNS Reagent: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.

Substrate: Add 38.5 mL deionized water for 96 T or 19.25 mL deionized water for 48 T before use, then shake upside down several times and heat to dissolve. This solution can be stored at 4℃ for 4 weeks. If there is precipitation, it can be heated at 70℃ to dissolve.

Standard: Containing 10 mg anhydrous glucose, add 1 mL deionized water to dissolve before use. The concentration is 10 mg/mL, which could be stored at 4℃ for 2 weeks.

Standard curve setting: Dilute 10 mg/mL Standard with deionized water to 1, 0.5, 0.25, 0.125, 0.0625, 0.0313, 0.0156 mg/mL standard solution as shown in the table below.

Num.	Volume of Standard (μL)	Volume of Deionized Water (μL)	The Concentration of Standard（mg/mL）
Std.1	40 μL 10 mg/mL	360	1
Std.2	200 μL of Std.1 (1 mg/mL)	200	0.5
Std.3	200 μL of Std.2 (0.5 mg/mL)	200	0.25
Std.4	200 μL of Std.3 (0.25 mg/mL)	200	0.125
Std.5	200 μL of Std.4 (0.125 mg/mL)	200	0.0625
Std.6	200 μL of Std.5 (0.0625 mg/mL)	200	0.0313
Std.7	200 μL of Std.6 (0.0313 mg/mL)	200	0.0156

Sample Preparation

Note: Fresh samples are recommended. If not assayed immediately, samples can be stored at -80℃ for one month.

1. Animal tissues: Weigh 0.1 g tissue, add 1 mL deionized water and homogenize. Pour the homogenate into a centrifuge tube, leave it at room temperature for 15 min, and shake it every 5 min to make it fully extracted. Centrifuge at 6,000 g for 10 min at room temperature. Aspirate the supernatant and add deionized water to make the volume up to 10 mL. Shake well to obtain the amylase stock solution. Take 1 mL of the above amylase stock solution, add 4 mL of deionized water, and shake well to form the amylase diluent, which is used to determine the total activity of (α+β) amylase.
2. Plant tissues: Weigh 0.1 g tissue, add 1 mL deionized water and mash. Ultrasonic break 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Leave it at room temperature for 15 min, and shake it every 5 min to make it fully extracted. Centrifuge at 6,000 g for 10 min at room temperature. Aspirate the supernatant and add deionized water to make the volume up to 10 mL. Shake well to obtain the amylase stock solution. Take 1 mL of the above amylase stock solution, add 4 mL of deionized water, and shake well to form the amylase diluent, which is used to determine the total activity of (α+β) amylase.
3. Liquid samples such as plasma, serum and saliva: Tested directly. It is recommended to determine the appropriate dilution factor in a pre-experiment. The properly diluted liquid sample is the amylase stock solution. Take 1 mL of the above amylase stock solution, add 4 mL of deionized water, and shake well to form the amylase diluent, which is used to determine the total activity of (α+β) amylase.

Note: For animal tissues with high fat content, remove the upper layer of fat after centrifugation, and then take the supernatant. It will be better to quantify the total protein with Protein Quantification Kit (BCA Assay), Cat #: KTD3001, if the content is calculated by protein concentration.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 540 nm, visible spectrophotometer was returned to zero with deionized water. Preheat the water bath to 70℃.
2. Take 2 EP tubes for each sample and add 75 μL of amylase stock solution and amylase diluent, and then use boiling water bath heater for 5 min as α-amylase control tube and total amylase control tube.
3. Add the following reagents respectively into each EP tube:

Reagent	Determination of Standard Curve		Determination of α-amylase Activity		Determination of Total Amylase Activity	
	Standard Tube (μL)	Blank Tube (μL)	Test Tube (μL)	Control Tube (μL)	Test Tube (μL)	Control Tube (μL)
Stds.	75	0	0	0	0	0
Deionized Water	0	75	0	0	0	0
Amylase Stock Solution	0	0	75	75(Boiled Amylase Stock Solution)	0	0
Placed in a 70℃ water-bath for 15 min and allow to cool						
Amylase Diluent	0	0	0	0	75	75(Boiled Amylase Diluent)
Substrate	0	0	75	0	75	0
Accurately keep warm for 5 min in a 40℃ water bath						
DNS Reagent	150	150	150	150	150	150
Substrate	75	75	0	75	0	75

4. Mix well, placed in a boiling water bath for 5 min and allow to cool. Take out 200 μL to a 96-well plate or microglass cuvette. Then reading the values at 540 nm. Finally, calculate $\Delta A_{\alpha}=A_{Test\ \alpha}-A_{Control\ \alpha}$, $\Delta A_{Total}=A_{Test\ Total}-A_{Control\ Total}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.

Note: Each sample needs to be set separately for α-amylase activity control tube and total amylase activity control tube. Only one blank well needs to be detected. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If the sample's A_{Test} is higher than 2, please further dilute the sample with deionized water. Pay attention to multiply by the dilution factor when calculating the result. If the ΔA_{Test} is less than 0.005, the sample can be re-extracted and reduce the volume of deionized water.

Data Analysis

1. Drawing of standard curve

With the concentration of the standard Solution as the y-axis and the $\Delta A_{standard}$ as the x-axis, draw the standard curve. Substitute the ΔA_{α} into the equation to obtain the y_1 value (mg/mL). Substitute the ΔA_{Total} into the equation to obtain the y_2 value (mg/mL).

2. Calculate the activity of α-Amylase in sample

(1) Calculated by fresh weight of samples

Unit definition: 1 mg reducing sugar produced per min in 1 g tissue reaction system is defined as a unit of enzyme activity.

$\alpha\text{-Amylase (U/g)}=y_1\times V_{Sample}\div(W\times V_{Sample}\div V_{Total\ Sample})\div T\times n=\mathbf{2\times y_1\div W\times n}$

(2) By protein concentration

Unit definition: 1 mg reducing sugar produced per min in 1 mg tissue protein reaction system is defined as a unit of enzyme activity.

α-Amylase (U/mg prot)=$y_1 \times V_{Sample} \div (Cpr \times V_{Sample}) \div T \times n$**=0.2×$y_1$÷Cpr×n**

(3) Calculated by volume of liquid sample

Unit definition: 1 mg reducing sugar produced per min in 1 L liquid sample reaction system is defined as a unit of enzyme activity.

α-Amylase (U/L)=1,000×y_1÷T×n**=200×y_1×n**

3. Calculate the activity of total amylase in sample

(1) Calculated by fresh weight of samples

Unit definition: 1 mg reducing sugar produced per min in 1 g tissue reaction system is defined as a unit of enzyme activity.

Total amylase (U/g)=$5 \times y_2 \times V_{Sample} \div (W \times V_{Sample} \div V_{Total\ Sample}) \div T \times n$**=10×$y_2$÷W×n**

(2) By protein concentration

Unit definition: 1 mg reducing sugar produced per min in 1 mg tissue protein reaction system is defined as a unit of enzyme activity.

Total amylase (U/mg prot)=$5 \times y_2 \times V_{Sample} \div (Cpr \times V_{Sample}) \div T \times n$**=$y_2$÷Cpr×n**

(3) Calculated by volume of liquid sample

Unit definition: 1 mg reducing sugar produced per min in 1 L liquid sample reaction system is defined as a unit of enzyme activity.

Total amylase (U/L)=5×1,000×y_2÷T×n**=1,000×y_2×n**

4. Calculate the activity of β-amylase in sample

(1) Calculated by fresh weight of samples

Unit definition: 1 mg reducing sugar produced per min in 1 g tissue reaction system is defined as a unit of enzyme activity.

β-amylase (U/g)=total amylase (U/g)-α-amylase (U/g)=(10×y_2÷W×n)-(2×y_1÷W×n)**=(10×y_2-2×y_1)÷W×n**

(2) By protein concentration

Unit definition: 1 mg reducing sugar produced per min in 1 mg tissue protein reaction system is defined as a unit of enzyme activity.

β-amylase (U/mg prot)=total amylase (U/mg prot)-α-amylase (U/mg prot)=(y_2÷Cpr×n)-(0.2×y_1÷Cpr×n)**=(y_2-0.2×y_1)÷Cpr×n**

(3) Calculated by volume of liquid sample

Unit definition: 1 mg reducing sugar produced per min in 1 L liquid sample reaction system is defined as a unit of enzyme activity.

β-amylase (U/L)=total amylase (U/L)-α-amylase (U/L)=(1,000×y_2×n)-(200×y_1×n)**=(1,000×y_2-200×y_1)×n**

Where: V_{Sample}: sample volume added, 0.075 mL; W: sample weight, g; $V_{Total\ Sample}$: total volume of sample, 10 mL; T: reaction time, 5 min; n: dilution factor; Cpr: sample protein concentration, mg/mL; 1,000: 1 L=1,000 mL; 5: total amylase dilution factor.

Typical Data

Typical standard curve

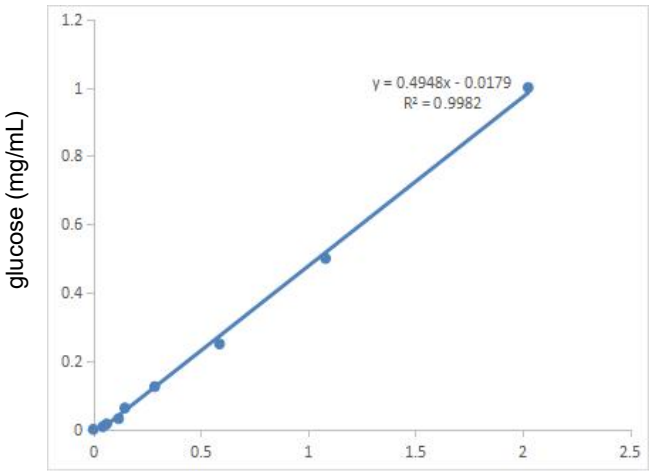

Figure1. Standard Curve for glucose.

Recommended Products

Catalog No.	Product Name
KTB1300	CheKine™ Micro Glucose Assay Kit
KTB1310	CheKine™ Micro Glucose Oxidase Activity (GOD) Assay Kit
KTB1320	CheKine™ Micro Plant Soluble Sugar Assay Kit
KTB1330	CheKine™ Micro Blood Glucose Assay Kit
KTB1340	CheKine™ Micro Glycogen Assay Kit
KTB1350	CheKine™ Micro Total Carbohydrate Assay Kit
KTB1360	CheKine™ Micro Reducing Sugar (RS) Assay Kit
KTB1370	CheKine™ Micro α-Amylase Activity Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.