α-Amylase Activity Assay Kit (EPS-G7 Colorimetric Method) (animal tissue, bacteria/cells, and Serum/plasma).

Note: It is necessary to predict 2-3 large difference samples before the formal determination.

Operation Equipment: Spectrophotometer/Microplate Reader

Catalog Number: JYM006CHS

Size: 100T/48S

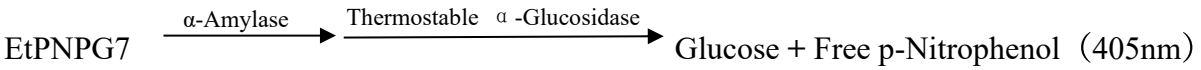
Product composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact our staff in time.

Reagent name	Size	Preservation Condition
Extract solution	Liquid 70 mL×1	2-8°C
Reagent I	Liquid 3 mL ×1	2-8°C
Reagent II	Liquid 5.5 mL ×1	2-8°C
Reagent III	Liquid 35 mL ×1	2-8°C
96 well plate (Cat#YA051)	96 well×1	-
Sealing film（Cat#YA0249）	2 pieces	-

Product Description:

α-amylase (α-amylase, EC 3.2.1.1), also known as 1,4-α-D-glucan-glucan hydrolase, can hydrolyze the α-1,4-glycosidic bonds inside starch, with the hydrolysis products being dextrin, oligosaccharides, and monosaccharides. α-amylase is mainly used to hydrolyze starch to produce maltose, glucose, syrup, and other products, as well as to produce dextrin, beer, yellow wine, alcohol, soy sauce, vinegar, fruit juice, and MSG.

Under the action of α-amylase, 4,6-O-ethyl-α-4-nitrophenyl-maltasacchaside (EtPNPG7). Hydrolysis produces p-nitrophenol triose malt (p-nitrophenol triose malt)/ tetrasaccharide and maltotriose /tetrasaccharide. The resulting substances are processed under the action of heat-resistant α-glucosidase (α-glucosidase) into glucose and yellow p-nitrophenol (p-nitrophenol/pNP). The higher the activity of α-amylase in the sample, the more yellow p-nitrophenol catalyzed is produced. By measuring the absorbance of p-nitrophenol at 405nm, the activity of α-amylase in the sample is finally calculated.



Reagents and Equipment Required but Not Provided:

Spectrophotometer/microplate Reader, micro glass cuvette/96 well plate, balance, centrifuge, water bath, mortar / homogenizer/cell ultrasonic crusher, vortex mixer/oscillator, ice and distilled water.

Operation procedure:

I. Sample preparation (The sample size to be tested can be adjusted appropriately, and the specific proportion can be referred to the literature.)

1. Animal tissue: Add extract solution according to the ratio of tissue mass (g): Extract solution volume (mL) = 1:5~10 (it is recommended to weigh 0.1 g sample and add 1.0 mL of extract solution), after ice bath homogenization, shake at 40°C for 20 minutes, then 12000g and centrifuge at 4°C for 10 minutes, take supernatant and placed on the ice for test.

2. Bacteria or cells: The ratio of bacteria/cell amount (10^6): the volume of extract solution (mL) is 5~10:1 (it is suggested to take about 5×10^6 bacteria/cell and add 1 mL of extract solution), ultrasonic shattering in ice bath (power 200W, ultrasound for 3 seconds, interval 7 seconds, total time 3 minutes); Shake and extract at 40°C for 20 minutes, 12000 g centrifuge at 4°C for 10 minutes, and place supernatant on ice for testing.
3. Serum (plasma): Directly to be tested. (If the sample is cloudy, centrifuge and use the supernatant for measurement)

II. Determination procedure

1、The Spectrophotometer/microplate reader is preheated for more than 30 minutes, the wavelength is adjusted to 405nm, and distilled water is used for zeroing.

2、Operation Table: Add the following reagents sequentially to a 1.5 mL EP tube

Reagent Name (μL)	Test tube	Control tube
Reagent I	50	-
Reagent II	50	50
Distilled water	-	50
Incubate at 40°C for 5 minutes		
Extract solution	80	80
Sample	20	20
React at 40°C for 20 minutes		
Reagent III	300	300
Mix thoroughly, pipette 200μL into a micro glass cuvette or 96 well plate to measure the absorbance at 405nm, record as A_T (Test tube) and A_C (Control tube), and calculate $\Delta A = A_T - A_C$.		

III. α-Amylase activity calculation formula

A. 96-Well flat-bottom plates

1. Calculated by sample protein concentration:

Unit definition: In a reaction system, producing 1 μmol of p-nitrophenol per minute per milligram of protein is defined as one unit of enzyme activity.

α - Amylase activity (U/mg prot) = $[\Delta A\div(\varepsilon\times d)\times V_{RV}\times10^6]\div(V_S\div V_E\times Cpr)\div T\times F = 0.111\times\Delta A\div Cpr\times F$

2. Calculated by sample mass:

Unit definition: In a reaction system, producing 1 μmol of p-nitrophenol per minute per gram of tissue is defined as one unit of enzyme activity.

α - Amylase activity (U/g mass) = $[\Delta A\div(\varepsilon\times d)\times V_{RV}\times10^6]\div(V_S\div V_E\times W)\div T\times F = 0.111\times\Delta A\div W\times F$

3. Calculated by liquid volume:

Unit definition: In a reaction system, producing 1 μmol of p-nitrophenol per minute per milliliter of liquid is defined as one enzyme activity unit.

α - Amylase activity(U/mL) = $[\Delta A\div(\varepsilon\times d)\times V_E\times10^6]\div V_S\div T\times F = 0.111\times\Delta A\times F$

4. Calculated by the number of bacteria or cells

Unit definition: In a reaction system, producing 1 μmol of p-nitrophenol per minute per 10^4 bacteria/cells is defined as one enzyme activity unit.

α - Amylase activity (U/10^4 cells) = $[\Delta A\div(\varepsilon\times d)\times V_{RV}\times10^6]\div(N\div V_E\times V_S)\div T\times F = 0.111\times\Delta A\div N\times F$

V_{RV}: Total volume of reaction system, 5×10^{-4}L;

ε: Molar extinction coefficient of p-nitrophenol, 18.8×10^3 L/mol/cm;
d: 96 well plate light path, 0.6 cm;
V_S: Add sample volume, 0.02mL;
V_E: Extract solution volume, 1mL;
T: Reaction time, 20 min;
W: Sample mass, g;
Cpr: Sample protein concentration, mg/mL;
N: Total number of cells or bacteria, count by 10^4;
10^6: Unit conversion factor, 1 mol = 10^6 μmol;
F: Sample dilution factor.

B. Micro glass cuvette

1. Calculated by sample protein concentration:

Unit definition: In a reaction system, producing 1 μmol of p-nitrophenol per minute per milligram of protein is defined as one unit of enzyme activity.

α - Amylase activity (U/mg prot) = $[\Delta A\div(\varepsilon\times d)\times V_{RV}\times10^6]\div(V_S\div V_E\times Cpr)\div T\times F = 0.0665\times\Delta A\div Cpr\times F$

2. Calculated by sample mass:

Unit definition: In a reaction system, producing 1 μmol of p-nitrophenol per minute per gram of tissue is defined as one unit of enzyme activity.

α - Amylase activity (U/g mass) = $[\Delta A\div(\varepsilon\times d)\times V_{RV}\times10^6]\div(V_S\div V_E\times W)\div T\times F = 0.0665\times\Delta A\div W\times F$

3. Calculated by liquid volume:

Unit definition: In a reaction system, producing 1 μmol of p-nitrophenol per minute per milliliter of liquid is defined as one enzyme activity unit.

α - Amylase activity(U/mL) = $[\Delta A\div(\varepsilon\times d)\times V_{RV}\times10^6]\div V_S\div T\times F = 0.0665\times\Delta A\times F$

4. Calculated by the number of bacteria or cells

Unit definition: In a reaction system, producing 1 μmol of p-nitrophenol per minute per 10^4 bacteria/cells is defined as one enzyme activity unit.

α - Amylase activity(U/10^4 cells) = $[\Delta A\div(\varepsilon\times d)\times V_{RV}\times10^6]\div(N\div V_E\times V_S)\div T\times F = 0.0665\times\Delta A\div N\times F$

V_{RV}: Total volume of reaction system, 5×10^{-4}L;
ε: Molar extinction coefficient of p-nitrophenol, 18.8×10^3 L/mol/cm;
d: Optical path length of micro glass cuvette, 1 cm;
V_S: Add sample volume, 0.02mL;
V_E: Extract solution volume, 1mL;
T: Reaction time, 20 min;
W: Sample mass, g;
Cpr: Sample protein concentration, mg/mL;
N: Total number of cells or bacteria, count by 10^4;
10^6: Unit conversion factor, 1 mol = 10^6 μmol;
F: Sample dilution factor.

Notes:

1. When the sample ΔA >1, the sample can be appropriately diluted with **extract solution** before measurement; When ΔA < 0.01, increase the sample volume while decreasing the volume of **extract solution** or **reagent III** before measurement, and be sure to modify the calculation formula accordingly.

Experimental example:

1、The test results for different types of samples are as follows:

Sample	Mass/volume	Dilution factor	A_T	A_C	ΔA	α-Amylase	Unit
Rat kidneys	0.1g	1	1.092	0.289	0.803	0.891	U/g mass
Rat spleen	0.1g	1	1.097	0.778	0.319	0.354	U/g mass
Rat plasma	0.02mL	20	0.804	0.050	0.754	1.674	U/mL

Note: 1. The above experiment uses a 96 well plate for measurement.