Amylase Assay Kit

(Cat/No.:BC041 Size:100T/ 96S)

1. Principle (Starch-Iodine Colorimetric Method)

Amylase catalyzes the hydrolysis of starch and the unreacted starch can react with iodine solution to generate blue complex. The concentration of unreacted starch can be determined spectrophotometrically and thus the amylase activity can be calculated.

2. Compositions and Preparation (The kit is valid for 6 months)

Reagent I: Substrate buffer solution (0.4 mg/mL): 60 mL × 1 bottle, store at 4℃

Reagent II: Iodine stock solution (0.1 mol/L): 7 mL × 1 bottle, store at 4℃ away from light. Dilute the iodine stock solution with doubledistilled water at a ratio of 1:9. Prepare immediately before use and store at 4℃ away from light.

3. Required Instruments and Reagents

Visible spectrophotometer with 1 cm optical path cuvettes,Vortex mixer,37℃ water bath,Double-distilled water (or distilled water),Normal saline,Protein assay reagent.

(for tissue or cell samples, available from our company: Cat. No. BC016)

4. Operation Steps

1. Sample Pretreatment

Serum (plasma) or digestive juice: Use directly.

Animal tissue:

Weigh the tissue, add normal saline at a weight-to-volume ratio of 1 (g):9 (mL), perform mechanical homogenization, centrifuge at 3500 rpm for 10 minutes, and take the supernatant (i.e., 10% homogenate supernatant) for testing (the protein concentration of the homogenate supernatant needs to be determined using the protein assay kit, Cat. No. BC016, available from our company).

Plant tissue:

Take the plant tissue, rinse surface contaminants with clean water, blot dry surface water, cut into pieces, put into a mortar, add liquid nitrogen, grind quickly into powder, and transfer to a sealable container (such as a centrifuge tube, self-sealing bag, etc.); weigh the plant powder, add phosphate buffer (0.1 M, pH 7.0-7.4) at a weight-to-volume ratio of 1 (g):4 (mL), place in a grinder for grinding (50 Hz, 30 seconds/time, run 2-3 times), take out and centrifuge at 3500 rpm for 10 minutes, and take the supernatant for testing.

Cultured cells: Take the prepared cell suspension, centrifuge at 1000 rpm for 10 minutes, discard the supernatant, and retain the cell pellet;

wash 1-2 times with isotonic buffer (recommended: 0.1 mol/L phosphate buffer, pH 7.0-7.4), centrifuge at 1000 rpm for 10 minutes again, discard the supernatant, and retain the cell pellet; add 0.2-0.3 mL of homogenization medium (recommended: 0.1 mol/L phosphate buffer, pH 7.0-7.4 or normal saline) for homogenization, perform ultrasonic disruption or manual homogenization under ice-water bath conditions, centrifuge the prepared homogenate at 3500 rpm for 10 minutes, and take the supernatant for testing.

Note: The dilution ratio varies for different samples, ranging from dozens to hundreds or even thousands of times, especially for chyme samples with high AMS content.

2. Operation Table

Compositions (ml)	Sample	Blank
Reagent I (pre-warmed at 37℃ for 5 minutes)	0.5	0.5
Sample	0.1	
Mix thoroughly and warm the mixture in a water bath at 37°C for 7.5 min.		
Iodine working solution (ml)	0.5	0.5
Double-distilled water (ml)	3.0	3.1
Mix and zero the 1cm path length cuvette at 660 nm with DDW. Record the optical density (OD) of each tube.		

Note: Pre-measurement can be done to determine the amount of reagent I added so that the absolute OD value (OD_{Blank}-OD_{Sample}) is in the range of 0.05-0.15.

5. Calculation and Examples

1. Calculation and Example for Serum (Plasma)

① Unit Definition

One unit (U) is defined as the amount of AMS in 100 ml of serum (plasma) that hydrolyzes 10 mg of starch when reacting with the substrate at 37℃ for 30 minutes.

② Calculation Formula

$$AMS\ activity\ (U/100mL) = \frac{(Blank\ OD - Assay\ OD) \times C_{substrate} \times V_{substrate} \times 30 \times N}{T \times V_{sample} \times 10}$$

- $C_{substrate}$: Concentration of substrate solution, 0.4 mg/mL;
- $V_{substrate}$: Volume of substrate solution added, 0.5 mL;
- T: Reaction time, 7.5 min;
- V_{sample}: Volume of sample added, 0.1 mL;
- N: Dilution ratio of sample before testing.

③ Calculation Example

A serum sample was diluted 10 times with normal saline, and0.1 mL of the diluted sample was used for AMS determination.The OD value of the assay tube was 0.308, and the OD

value of the blank tube was 0.383.

The calculation of AMS activity:

$$\text{AMS activity } (U/dl) = \frac{0.383-0.308}{0.383} \times \frac{0.4\times0.5}{10} \times \frac{30}{7.5} \times \frac{100}{0.1} \times 10 = 156.0830\ U/dl$$

2.Calculation and Examples for Tissues or Cells

① Unit Definition

One amylase activity unit is defined as the amount of enzyme that hydrolyzes 10 mg of starch p er milligram of protein in tissue when reacting with the substrate at 37℃ for 30 minutes.

② Calculation Formula

$$AMS\ activity\ (U/mgprot) = \frac{(Blank\ OD - Assay\ OD)\times C_{substrate}\times V_{substrate}\times 30}{T\times V_{sample}\times 10\times C_{pr}}$$

- $C_{substrate}$: Concentration of substrate solution, 0.4 mg/mL;
- $V_{substrate}$: Volume of substrate solution added, 0.5 mL;
- T: Reaction time, 7.5 min;
- V_{sample}: Volume of sample added, 0.1 mL;
- C_{pr}: Protein concentration of tissue homogenate, mgprot/mL (prot = protein).

③ Calculation Examples

Example 1: 0.1 mL of 1% rat lung homogenate was used for AMS determination. The OD value of the assay tube was 0.275,
the OD value of the blank tube was 0.383, and the protein concentration of the 1% rat lung ho mogenate was 0.5677 mgprot/ml. The calculation result is as follows:

$$\text{AMS activity U/mgprot} = \frac{0.383-0.275}{0.383} \times \frac{0.4\times0.5}{10} \times \frac{30}{7.5} \div (0.1\times0.5677) = 0.3973\text{U/mgprot}$$

Example 2: Take 0.1 mL of 2.5% fish intestine homogenate for AMS determination. The OD value of the test tube is 0.274, and the OD value of the blank tube is 0.388. The protein concentration of the 2.5% fish intestine homogenate is 0.5960 mg prot/mL. The calculated result is:

$$\text{AMS activity U/mgprot} = \frac{0.388-0.274}{0.388} \times \frac{0.4\times0.5}{10} \times \frac{30}{7.5} \div (0.1\times0.596) = 0.3944\text{U/mgprot}$$

6. NOTICE

1. After completing the procedure according to the protocol, the blank tube should exhibit a blue-brown color (leaning toward brown), while the test tube should show a slightly lighter shade. Note that if the test tube appears yellow (or exhibits excessively low absorbance), the sample concentration is likely too high. Dilute the sample further before retesting; otherwise, calculated results will be underestimated.

2. α-Amylase activity varies significantly across different samples, with chyme samples exhibiting the greatest variation (requiring dilutions ranging from tens to thousands of times). Note that mouse and rat serum samples require 50-100-fold dilution prior to testing.

Appendix I: Rat Serum Alpha-Amylase Concentration Curve

1. Pretreatment

Dilute rat serum with physiological saline to different concentrations: 2-fold, 4-fold, 8-fold, 16-fold, 32-fold, 64-fold, 128-fold, and 256-fold. Take 0.1 mL samples for testing.

2. Procedures

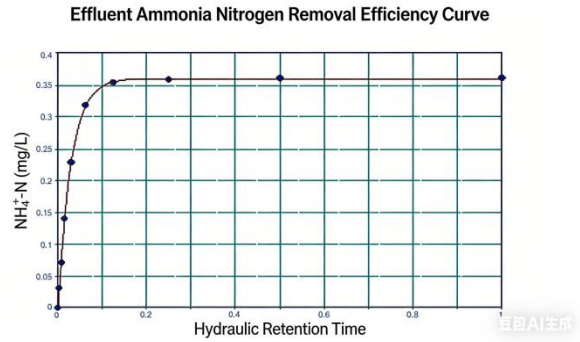
Compositions (ml)	Sample	Blank
Reagent I (pre-warmed at 37℃ for 5 minutes)	0.5	0.5
Sample	0.1	
Mix thoroughly and warm the mixture in a water bath at 37 °C for 7.5 min.		
Iodine working solution (ml)	0.5	0.5
Double-distilled water (ml)	3.0	3.1
Mix and zero the 1cm path length cuvette at 660 nm with DDW. Record the optical density (OD) of each tube.		

3. Results

Conc./Initial Conc.	OD Measured	Absolute OD
Blank	0.369	0.000
1	0.008	0.361
0.5	0.008	0.361
0.25	0.010	0.359
0.125	0.015	0.354
0.0625	0.050	0.319
0.03125	0.140	0.229
0.015625	0.227	0.142
0.007813	0.297	0.072
0.003906	0.336	0.033

4. Standard Curve

Standard Curve



In conclusion, the optimal sample concentration selected is 0.07813, which corresponds to a 128-fold dilution.

Appendix II: Standard Curve of Rat Tissue Establishment

1. Pretreatment

Weigh the tissue precisely and add saline with the ratio of 1 g tissue to 9 ml saline. Homogenize the mixture in an ice water bath and centrifuge the homogenate at 2,500 rpm for 10 min. Extract and dilute the supernatant to 2, 4, 8, 16, 32, 64, 128, 256 times of its initial volume respectively.

2. Procedures

Compositions (ml)	Sample	Blank
Reagent I (pre-warmed at 37℃ for 5 minutes)	0.5	0.5
Sample	0.1	
Mix thoroughly and warm the mixture in a water bath at 37°C for 7.5 min.		
Iodine working solution (ml)	0.5	0.5
Double-distilled water (ml)	3.0	3.1
Mix and zero the 1cm path length cuvette at 660 nm with DDW. Record the optical density (OD) of each tube.		

3. Results

Percentage Conc.	OD Measured	Absolute OD
Blank	0.368	
10.000%	0.034	0.335
5.000%	0.113	0.256
2.500%	0.168	0.200
1.250%	0.234	0.135
0.625%	0.287	0.081
0.313%	0.326	0.042
0.156%	0.353	0.015
0.078%	0.367	0.001

4. Standard Curve