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Serum α-Amylase (AMY) Activity Assay Kit (Iodine-starch colorimetry)

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

Detection Equipment: Spectrophotometer/Microplate reader

Catalog Numder: AK0960-100T-48S

Size: 100T/48S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. .

Reagent name	Size	Preservation Condition
Reagent I	Powder×1	2-8℃
Reagent II	Liquid 30mL×1	2-8℃
Reagent III A	Powder×1	2-8℃
Reagent III B	Powder×1	2-8℃
Standard	Powder×1	2-8℃

Solution Preparation:

1. **Reagent I:** Add 12.5mL of Reagent II when the solution will be used. The solution is placed in water at room temperature. Heat to boil, stir continuously until the powder dissolves completely. Store at 2-8℃ for one month.
2. **Reagent III:** Pour Reagent III A to Reagent III B, make up to 10 mL with distilled water. Store at 4 ℃ in the dark for one month.
3. **Standard:** 10 mg of starch. Add 10 mL of Reagent II to form 1 mg/mL starch standard solution when the solution will be used. The solution is placed in water at room temperature. Heat to boil, stir continuously until the powder dissolves completely. Store at 2-8 ℃ for one month.

Product Description:

Serum amylase (AMY) belongs to α-amylase, which hydrolyzes α-1,4 glycosidic bonds inside polysaccharide molecules in a random manner to generate a mixture of oligosaccharides, maltose and glucose. AMY is mainly secreted by the salivary glands and pancreas, and a small amount of it is secreted by organs such as the proximal duodenum, lungs, uterus, and breast during lactation.

Amylase catalyzes the hydrolysis of α-1,4 glycosidic bonds in starch molecules to produce glucose, maltose, dextrin, etc. Iodine can be combined with starch that is not hydrolyzed by amylase to form a complex with a characteristic absorption peak at 570 nm. The depth can calculate the unit of amylase activity.

Required material:

Spectrophotometer/microplate reader, water bath/constant temperature incubator, desktop centrifuge,

adjustable pipette, mortar/homogenizer, 1 mL glass cuvette, distilled water.

Operation procedure:

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

Take 40μL of serum and 160μL of distilled water and mix (dilute the serum 5 times), divide it into 2 tubes of 100μL as the measurement tube and the control tube. If the measured value after the experiment is too large or too small, you can adjust the dilution ratio (for example, if the value is too small, you can mix 80μL of serum with 120μL of distilled water to dilute the serum 2.5 times)

II. Detection

1. Preheat spectrophotometer/microplate reader for 30 minutes, adjust wavelength to 570 nm, set zero with distilled water.

2. Dilute the 1 mg/mL starch standard solution with distilled water to 0.5, 0.4, 0.2, 0.1, 0.05, 0.025, 0.0125, 0.00625mg/mL.

3. Add each reagent in turn according to the operation table

Reagent Name (μL)	Test tube (A_T)	Contrast tube (A_C)	Blank tube (A_{B1})	Standard tube (A_S)	Standard blank tube (A_{B2})
Serum diluent	100	100	-	-	-
Distilled water	-		100	-	100
Standard solution	-	-		100	-
Reagent I	100	-	100	-	-
Reagent II		100	-	100	100
Incubate in 37℃ thermostat water bath for 10 minutes.					
Reagent III	50	50	50	50	50

Mix well, pipette 200μL into a micro glass cuvette or 96-well plate, measure the absorbance at 570 nm **in 15 min**, recorded as A_T, A_C, A_{B1}, A_S, and A_{B2} respectively from left to right. $\Delta A_T = (A_{B1}-A_{B2})-(A_T-A_C)$, $\Delta A_S = A_S-A_{B2}$.

III. Calculation:

1. Create standard curve

Using the concentration of standard solution as x axis and ΔA_S as y axis create standard curve, obtain equation y=kx+b. Put ΔA into the equation and obtain the x (mg/mL).

2. Calculation of α-amylase activity

Definition of unit: One unit is defined as an enzyme activity that per milligram of tissue protein catalyze the hydrolyze of 1 mg of starch per minute.

α-amylase activity(U/mL)= $x \times V_S \div V_S \div T \times F = 0.1 \times x \times F$

V_S: The volume of sample added to reaction system, 0.1mL;

T: Reaction time, 10 minutes;

F: Dilution factor

Note:

1. When the ΔA measurement is greater than 1.5, the sample can be appropriately diluted and measured.
2. Measure the absorbance within 15 minutes after the reaction is completed

Experimental Examples:

Take 40μL of bovine serum and 160μL of distilled water and mix (the serum is diluted 5 times), and then follow the determination steps to calculate $\Delta A = (A_{B1}-A_{B2})-(A_T-A_C)$ =（1.409-0.067）-（1.297-0.058）=0.103, standard curve y =2.6047x-0.0165, calculate x=0.0459, calculate activity according to the formula:

AMY (U/mL)=0.1×x×F =0.1×0.0459×5 =0.0230 U/mL