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

Note: Take two or three different samples for prediction before test.

Detection instrument: Spectrophotometer/Microplate reader

Cat No: BC5055

Size: 100T/48S

Components:

Reagent	Size	Storage Condition
Reagent I	Powder×1	4℃
Reagent II	30mL×1	4℃
Reagent III A	Powder×1	4℃
Reagent III B	Powder×1	4℃
Standard	Powder×1	4℃

Preparation of the solution:

1. Reagent I：Add 12.5mL of Reagent III 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 4 ℃ for one month.
2. Reagent III：Pour Reagent III A to Reagent III B, make up to 20 mL with distilled water. Store at 4 ℃ in the dark for one month.
3. Standard: Powder×1, 10 mg of starch. Add 10 mL of Reagent III to form 1 mg/mL starch standard solution when the solution will be used. Dissolve by shaking in a boiling water bath to prepare a 1 mg/mL starch standard solution. Store at 4 ℃ 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. α-amylase is acid-resistant and β-amylase is heat-resistant. According to the above characteristics, the activity of another amylase can be measured by passivating one of them.

Required material:

Spectrophotometer/microplate reader, water bath/ incubator, desktop centrifuge, transferpettor, mortar/ homogenizer, micro glass cuvette/96 well UV plate, mortar, distilled water.

Procedure:

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I. Sample extraction:

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_B)	Standard tube (A_{S1})	Standard blank tube (A_{S0})
Serum	100	100	-	-	-
Distilled water	-		100	-	100
Standard solution	-	-		100	-
Reagent I	100	-	100	-	-
Reagent II		100	-	100	100
Incubate in 40℃ 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_B, A_{S1}, and A_{S0} respectively from left to right. $\Delta A = A_B-(A_T-A_C)$, $\Delta A_S = A_{S1}-A_{S0}$.

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, 0.25 mL;

T: Reaction time, 10 minutes;

F: Dilution factor

Note:

When the measured absorbance value is greater than 1

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 $\Delta A = A_B-(A_T-A_C)$ =1.409-（1.297-0.058）=0.17, standard curve y = y=2.6422x-0.019, calculate x=0.07153, calculate activity according to the formula:

AMY (U/mL)=0.1×x×F =0.1×0.07153×5 =0.0356 U/mL

Related Products:

BC0700/BC0705	Starch Content Assay Kit
BC4260/ BC4265	Amylose Content Assay Kit
BC4270/ BC4275	Amylopectin Content Assay Kit
BC4570/ BC4575	α-Amylase Assay Kit(Iodine-starch colorimetry)
BC4580/ BC4585	β-Amylase Assay Kit(Iodine-starch colorimetry)

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