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α-L-Arabinofuranosidase (α-L-Af) Activity Assay Kit

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

Operation Equipment: Spectrophotometer/ Microplate reader

Catalog Number: AK0034

Size: 100T/48S

Components:

Extract solution: 100 mL×1. Storage at 4℃.

Reagent I: Powder×2. Storage at 4℃. Add 1.37 mL of distilled water to fully dissolve when the solution will be used. It is recommended to store the reagent at - 20℃ for one week after subpackage to avoid repeated freezing and thawing.

Reagent II:35 mL×1. Storage at 4℃.

Standard solution: 1mL×1, 5 μmol/mL p-nitrophenol solution.

Product Description

α-L-Arabinofuranosidase (α-L-Af) belongs to the family of glycosyl hydrolases, which can be hydrolyzed from the non-reducing ends of polymers containing arabinose residues such as arabinoxylan, arabinan and arabinose galactosan etc. to form an L-arabinose molecule. This enzyme plays an important role in the degradation of hemicellulose, the ripening and softening of fruits.

α-L-Af decomposes p-nitrophenyl-α-L-arabinofuranoside to produce p-nitrophenol, p-nitrophenol has a maximum absorption peak at 400 nm, which can be calculated by measuring the change in absorbance at 400 nm.

Reagents and Equipment Required but Not Provided

Spectrophotometer/ Microplate reader, centrifuge, water-bath, transferpettor, micro glass cuvette/96 well flat-bottom plate, mortar/homogenizer, ice and distilled water.

Procedure

1. Sample Extraction:

(1) Tissue sample:

According to the mass of the tissue (g): the volume of the extract solution (mL) is 1: 5- 10. Suggested 0. 1g of tissue with 1 mL of extract solution. Fully grind on ice, centrifuge at 15000 g and 4℃ for 10 min. Supernatant is placed on ice for test. (For stems, leaves, buds and other tissues of green plants, it is recommended to dilute the supernatant with the extract solution 5 times before testing; for tissues such as pulp, the supernatant can be directly tested or diluted after a corresponding multiple according to the predicted result)

(2) Bacteria or cells:

According to the number of cells (10^4): the volume of the extract solution (mL) is 500- 1000: 1. Suggest

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5 million with 1mL of extract Solution. Use ultrasonication to split bacteria or cells (power 300W, work time 3s， interval 7s， total time 3 min). centrifugated at 15000g and 4℃ for 10min. Supernatant is placed on ice for test.

(3) Liquid: direct measurement.

2. Determination steps and sample adding table:

a. Preheat spectrophotometer/ microplate reader more than 30 min, adjust wavelength to 400 nm and set zero with distilled water.

b. Dilute the 5 μmol/mL p-nitrophenol standard solution into 500, 250, 125, 62.5, 31.25, 15.625 nmol/mL standard solutions with the extraction solution for later use.

c. Operate according to the following table:

Reagent Name	Control tube (C)	Test tube (T)	Standard tube (S)	Blank tube (B)
Reagent I (μL)	-	40	-	-
Distilled water	40	-	40	100
sample (g)	60	60	-	-
Standard solution (μL)	-	-	60	-
Reacting for 30min at 37C in a water bath.			-	-
Reagent II (μL)	200	200	200	200

Mix well, take 200 μL of react solution into micro glass cuvette/96 well flat-bottom plate and record the absorption value A of each tube at 400 nm, calculate $\Delta A = A_T - A_C$, $\Delta A_S = A_S - A_B$

Calculation of α-L-Af activity:

1. Drawing of standard curve:

Taking the concentration of each standard solution as the x-axis and its corresponding ΔA_S as the y-axis, draw a standard curve to obtain a standard equation y=kx+b, bring ΔAS into the equation to get x (nmol/mL)

2. 2. Calculation of α-L-Af activity:

1) Tissue protein concentration

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the production of 1 μmol ofp-nitrophenol every mg of protein in the reaction system per hour.

α-L-Af Activity (U/mg prot)= $x \times V_E \div (Cpr \times V_E) \div T = 2x \div Cpr$

2) Tissue weight

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the production of 1 μmol ofp-nitrophenol every gram of tissue in the reaction system per hour.

α-L-Af Activity (U/g weight) = $x \times V_E \div W \div T = 2x \div W$

3) Bacteria or cultured cells

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the production of 1 μmol ofp-nitrophenol every 10^4 cells or bacteria in the reaction system per hour at.

C1 Activity (U/10^4 cell) =$x \times V_E \div$ cell amount $\div T = 2x \div$ cell number;

4) Liquid

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the production

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of 1 μmol ofp-nitrophenol every milliliter of liquid sample in the reaction system per hour.
α-L-Af Activity (U/mL) $=x\times V_S\div V_S\div T=2x$

V_S: Sample volume, 0.06 mL; Ve: Extract solution volume, 1 mL; Cpr: Supernatant sample protein concentration (mg/mL); T: Reaction time (min), 30 min; W: Sample weight, g; Cell amount: 10^4 cells as a unit.

Note

1. If the absorbance value is greater than 1.2, it is recommended to dilute the supernatant with extract solution.
2. Before the formal determination, 2-3 samples with large expected difference should be selected for prediction. If the concentration is too high, it is recommended to dilute the sample with the extract solution for an appropriate multiple before determination, and multiply the dilution multiple in the calculation formula; if the concentration is too low, it is recommended to increase the tissue mass during sample extraction.
3. After adding Reagent Ⅱ, it is recommended to read the OD value within 5 minutes.

Experimental example:

1. Take 0. 1g rye ear for sample treatment, take the supernatant to dilute 2 times, operate according to the determination steps, use 96 well plate to measure and calculate $\Delta A=A_T-A_C = 0.642-0.276 = 0.366$, bring in the standard curve y = 0.0024x-0.0045, calculate x = 154.375, calculate the enzyme activity according to the sample mass

α -L-AF activity (U/g mass) = 2x÷W×2(dilution ratio) =2×154.375÷0. 1×2(dilution ratio) = 6175 U/g mass.

Related products:

AK0061/AK0060 Hemicellulose Content Assay Kit
AK0205/AK0204 α-galactosidase (α -GAL) Activity Assay Kit
AK0203/AK0202 β-galactosidase (β -GAL) Activity Assay Kit
AK0209/AK0208 α-glucosidase (α -GC) Activity Assay Kit
AK0207/AK0206 β-glucosidase (β -GC) Activity Assay Kit