CheKine™ Micro α-L-Arabinofuranosidase (α-L-Af) Activity Assay Kit

Cat #: KTB4053 Size: 48 T/24 S 96 T/48 S

	Micro α-L-Arabinofuranosidase (α-L-Af) Activity Assay Kit		
REF	**Cat #:** KTB4053	LOT	**Lot #:** Refer to product label
	Applicable samples: Plant Tissue, Bacteria, Fungi, Liquid samples		
	Storage: Stored at -20℃ for 6 months, protected from light		

Assay Principle

α-L- arabinofuranosidase (α-L-AF) is a kind of glycosidases that can hydrolyze non-reducing arabinose residues, which can continuously dissociate neutral sugars such as arabinogalactan and arabinomannan in cell walls and promote the solubilization and degradation of pectin. Because arabinose is often lost during fruit ripening, it is of great significance to study the activity of arabinose in fruit ripening and softening. CheKine™ Micro α-L-Arabinofuranosidase (α-L-Af) Activity Assay Kit can be used to detect biological samples such as plant tissue, bacteria, fungi, or liquid samples. In the kit, α-L-Af decomposes p-nitrophenol arabinofuranoside to generate p-nitrophenol, which has the maximum absorption peak at 400 nm. α-L-Af activity is calculated by measuring the rising rate of absorbance.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	100 mL	100 mL×2	4℃
Reagent Ⅰ	Powder×1 vial	Powder×2 vials	-20℃, protected from light
Reagent Ⅱ	20 mL	40 mL	4℃
Standard	1 mL	1 mL	4℃, protected from light

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

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 400 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips, 1.5 mL EP tube
- Incubator, ice maker, freezing centrifuge
- Deionized water
- Homogenizer or mortar (for tissue samples)

Reagent Preparation

Extraction Buffer: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃.

Working Reagent Ⅰ: Prepared before use. Add 1.5 mL deionized water to each bottle to dissolve thoroughly. The remaining reagent can also be stored at -20℃ and protected from light for 2 weeks after aliquoting to avoid repeated freezing and thawing.

Reagent Ⅱ: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃.

Standard: Ready to use as supplied; 5 μmol/mL p-nitrophenol Standard. Equilibrate to room temperature before use; Store at 4℃, protected from light. Using 5 μmol/mL p-nitrophenol Standard, prepare standard curve dilution as described in the table:

Num.	Standard Volume (μL)	Extraction Buffer (μL)	Concentration (nmol/mL)
Std.1	100 μL of 5 μmol/mL Standard	900	500
Std.2	200 μL of Std.1 (500 μmol/mL)	200	250
Std.3	200 μL of Std.2 (250 μmol/mL)	200	125
Std.4	200 μL of Std.3 (125 μmol/mL)	200	62.5
Std.5	200 μL of Std.4 (62.5 μmol/mL)	200	31.25
Std.6	200 μL of Std.5 (31.25 μmol/mL)	200	15.625
Blank	0	400	0

Note: Always prepare fresh standards per use; Diluted Standard Solution is unstable and must be used within 4 h.

Sample Preparation

Note: We recommend that you use fresh samples. If not assayed immediately, samples can be stored at -80℃ for one month.When measuring, the temperature and time of thawing should be controlled. When thawing at room temperature, the sample should be thawed within 4 h.

1. Plant tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize or mortar on ice. Centrifuge at 15,000 g for 10 min at 4℃. Use supernatant for assay.
2. Bacteria or fungi: Collect 5×10^6 bacteria or fungi into the centrifuge tube, wash cells with cold PBS, discard the supernatant after centrifugation; add 1 mL Extraction Buffer to ultrasonically disrupt the bacteria or fungi 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 15,000 g for 10 min at 4℃. Use supernatant for assay.
3. Liquid samples: Test directly.

Note: If the protein concentration of the sample is need to determined, it is recommended to use Abbkine Cat #: KTD3001 Protein Quantification Kit (BCA Assay) to measure the protein concentration of the sample.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 400 nm. Visible spectrophotometer was returned to zero with deionized water.
2. Sample measurement. (The following operations are operated in 96-well plate or microglass cuvette **in turn**)

Reagent	Test Well (μL)	Control Well (μL)	Standard Well (μL)	Blank Well (μL)
Working Reagent Ⅰ	25	0	0	0
Deionized water	0	25	25	25
Extraction Buffer	0	0	0	45
Standard	0	0	45	0

Sample	45	45	0	0
Mix well quickly, Incubate for 30 min at 37℃				
Reagent II	130	130	130	130

3. Mix well, detect the absorbance at 400 nm **in 5 min**. The Blank Well is recorded as A_{Blank}, the Standard Well is marked as $A_{Standard}$, the Control Well is marked as $A_{Control}$, and the Test Well is marked as A_{Test}. Finally calculate $\Delta A_{Test}=A_{Test}-A_{Control}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.

Note: The Standard Well and Blank Well only need to be done once or twice, Each Test Well needs to be provided with a Control Well. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA_{Test} is less than 0.01, increase the sample quantity appropriately. If ΔA_{Test} is greater than $\Delta A_{Comtrol}$ of 500 μmol/mL, the sample can be appropriately diluted with Extraction Buffer, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

1. Drawing of standard curve

With the concentration of the standard solution as the x-axis and the $\Delta A_{Standard}$ as the y-axis, draw the standard curve and obtain the standard equation. The determination of ΔA_{Test} is substituted into the equation to get x (nmol/mL).

2. Calculation of the α-L-Af activity

(1) Calculated by protein concentration

Active unit definition: 1 nmol of p-nitrophenol is produced per h in 1mg tissue protein reaction system is defined as a unit of enzyme activity.

α-L-Af (U/mg prot)=$(V_{Sample}\times x)\div(V_{Sample}\times Cpr)\div T$**=2x÷Cpr**

(2) Calculated by fresh weight of samples

Active unit definition: 1 nmol of p-nitrophenol is produced per h in 1 g tissue reaction system is defined as a unit of enzyme activity.

α-L-Af (U/g fresh weight)=$(V_{Sample}\times x)\div(W\times V_{Sample}\div V_{Total\ sample})\div T$**=2x÷W**

(3) Calculated by bacteria or fungus

Active unit definition: 1 nmol of p-nitrophenol is produced per h in 10^4 bacteria or fungus reaction system is defined as a unit of enzyme activity.

α-L-Af ($U/10^4$)=$(V_{Sample}\times x)\div(n\times V_{Sample}\div V_{Total\ sample})\div T$**=2x÷n**

(4) Calculated by volume of liquid samples

Active unit definition: 1 nmol of p-nitrophenol is produced per h in 1 mL liquid samples reaction system is defined as a unit of enzyme activity.

α-L-Af (U/mL)=$(V_{Sample}\times x)\div V_{Sample}\div T$**=2x**

V_{Sample}: Added the sample volume, 0.045 mL; $V_{Total\ sample}$: Added the Extraction Buffer volume, 1 mL;T: Reaction time, 30 min=0.5 h; Cpr: sample protein concentration, mg/mL; W: Sample weight, g; n: Number of bacteria or fungus, calculated in units of ten thousand.

Typical Data

The following data are for reference only. And the experimenters need to test the samples according to their own experiments.

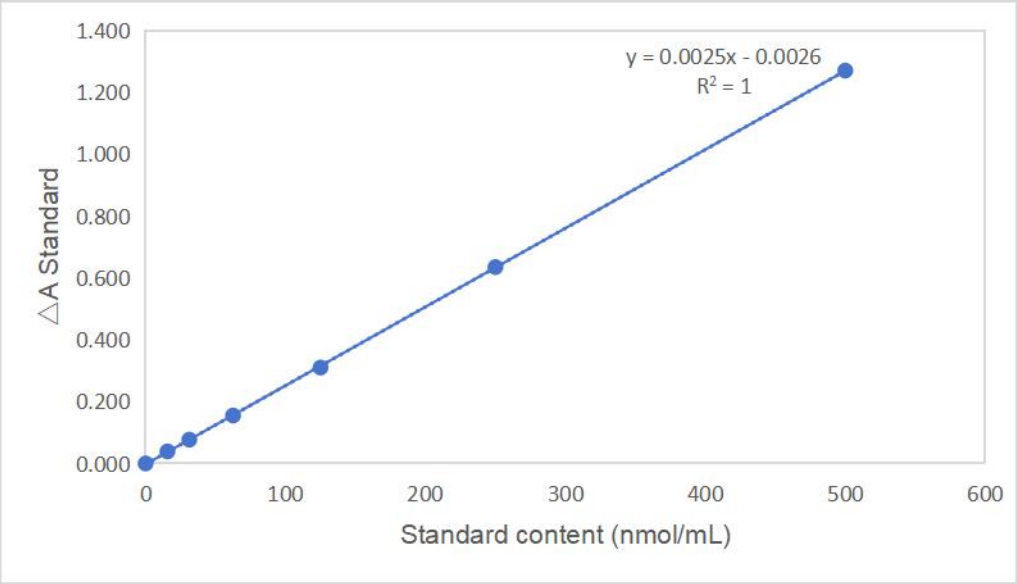

Figure 1. Standard curve of α-L-Af.

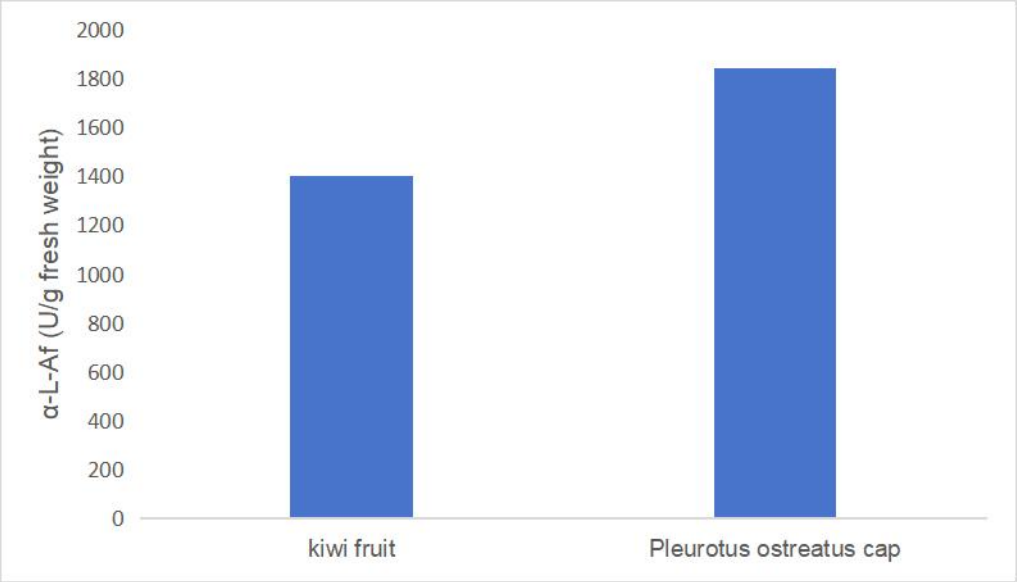

Figure 2. Determination of α-L-Af activity in kiwi fruit and pleurotus ostreatus cap intestine by this kit.

Recommended Products

Catalog No.	Product Name
KTB1150	CheKine™ Micro Peroxidase (POD) Activity Assay Kit
KTB1030	CheKine™ Micro Superoxide Dismutases (SOD) Activity Assay Kit
KTB1040	CheKine™ Micro Catalase (CAT) Content Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.