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CheKine™ Micro L-Galactono-1,4-Lactone Dehydrogenase (Gal LDH) Activity Assay Kit

Cat #: KTB1281 Size: 48 T/48 S 96 T/96 S

	Micro L-Galactono-1,4-Lactone Dehydrogenase (Gal LDH) Activity Assay Kit		
REF	**Cat #:** KTB1281	LOT	**Lot #:** Refer to product label
	Applicable sample: Plant tissues		
	Storage: Stored at -20℃ for 6 months, protected from light		

Assay Principle

L-galactose pathway is the main pathway of plant ascorbicacid (AsA) synthesis. L-galactose glucoside-1,4-lactone dehydrogenase (L-Galactono-1,4-Lactone Dehydrogenase, Gal LDH) is located in the mitochondrial membrane, responsible for the final step of the catalytic AsA biosynthesis in plants, is one of the key enzyme of the way. It plays a crucial role in the accumulation of AsA content in plants. CheKine™ Micro L-Galactono-1,4-Lactone Dehydrogenase (Gal LDH) Activity Assay Kit can detect plant tissues In this kit, Gal LDH catalyzed L-galactolactone reduction and oxidation of cytochrome C (Cyt c), and the reduced Cyt c had an absorption peak at 550 nm. Gal LDH activity was calculated by measuring the rate of increase of reduced Cyt c.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	60 mL	60×2 mL	4℃
Reagent Ⅰ	Powder×1 vial	Powder×1 vial	-20℃, protected from light
Reagent Ⅱ	Powder×1 vial	Powder×1 vial	-20℃, 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 550 nm
- 96-well microplate or microglass cuvette, precision pipettes, disposable pipette tips, 1.5 mL EP tube
- Water bath, cryogenic centrifuge
- Deionized water
- Mortar or homogenizer

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 9.6 mL deionized water for 48 T and 19.2 mL deionized water for 96 T to fully dissolve. The prepared reagent can also be stored at 4℃ and protected from light and used within 3 days.

Working Reagent Ⅱ: Prepared before use. Add 1.2 mL deionized water for 48 T and 2.4 mL deionized water for 96 T to fully dissolve. The prepared reagent can also be stored at 4℃ and protected from light and used within 3 days.

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.

Plant Tissues: weigh about 0.1 g sample, add 1mL of Extraction Buffer, homogenize in ice bath, and ultrasonically crush for 5 min (power 20% or 200 W, ultrasonic for 3 s, interval 7 s, 30 times), then centrifuge at 4℃ for 10 min at 13,000 g, and take the supernatant and put it on ice for testing.

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 550 nm, visible spectrophotometer was returned to zero with deionized water.
2. Working Reagent Ⅰ place at 25℃ incubation for 30 min.
3. Operation table (The following operations are operated in the 96-well microplate or microglass cuvette):

Reagent	Test Well (μL)	Blank Well (μL)
Sample	20	0
Deionized Water	0	20
Working Reagent Ⅰ	160	160
Working Reagent Ⅱ	20	20

4. Mix quickly, measure the absorbance value A_1 at 10 s at 550 nm, and the absorbance value A_2 at 130 s at 25℃ for 2 min. The Test Well is marked as A_{Test}, and the Blank Well is marked as A_{Blank}. Finally calculate $\Delta A=(A_{2Test}-A_{1Test})-(A_{2Blank}-A_{1Blank})$.

Note: The Blank Well only need to be done 1-2 times. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA is less than 0.01, increase the sample quantity appropriately. If ΔA is greater than 0.8, 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.

Calculation of Gal LDH activity:

A. 96-well plates calculation formula as below

(1) Calculated by protein concentration

Active unit definition: At 25℃, the reduction of 1 nmol of Cyt c per milligram of protein per min was defined as one unit of enzyme activity.

Gal LDH (U/mg prot)=$[\Delta A\times V_{Total}\div(\varepsilon\times d)\times 10^9]\div(V_{Sample}\times Cpr)\div T$**$=578.03\times\Delta A\div Cpr$**

(2) Calculated by fresh weight of samples

Active unit definition: At 25℃, the reduction of 1 nmol of Cyt c per gram tissue per min was defined as one unit of enzyme activity.

Gal LDH (U/g fresh weight)=[ΔA×V_{Total}÷(ε×d)×10^9]÷(W×V_{Sample}÷$V_{Total\ sample}$)÷T**=578.03×ΔA÷W**

V_{Total}: total reaction volume, 0.2 mL=0.0002 L; ε: Cyt c molar extinction coefficient, 17.3×10^3 L/mol /cm; d: the light path of the 96-well plate, 0.5 cm; 10^9: 1 mol=1×10^9 nmol; V_{Sample}: sample volume added, 20 μL=0.02 mL; $V_{Total\ sample}$: added Extraction Buffer volume, 1 mL; T: reaction time, 2 min; Cpr: sample protein concentration, mg/mL; W: weight of sample, g.

B. Microglass cuvette calculation formula

The optical diameter d: 0.5 cm in the above calculation formula can be adjusted to d: 1 cm for calculation.

Typical Data

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

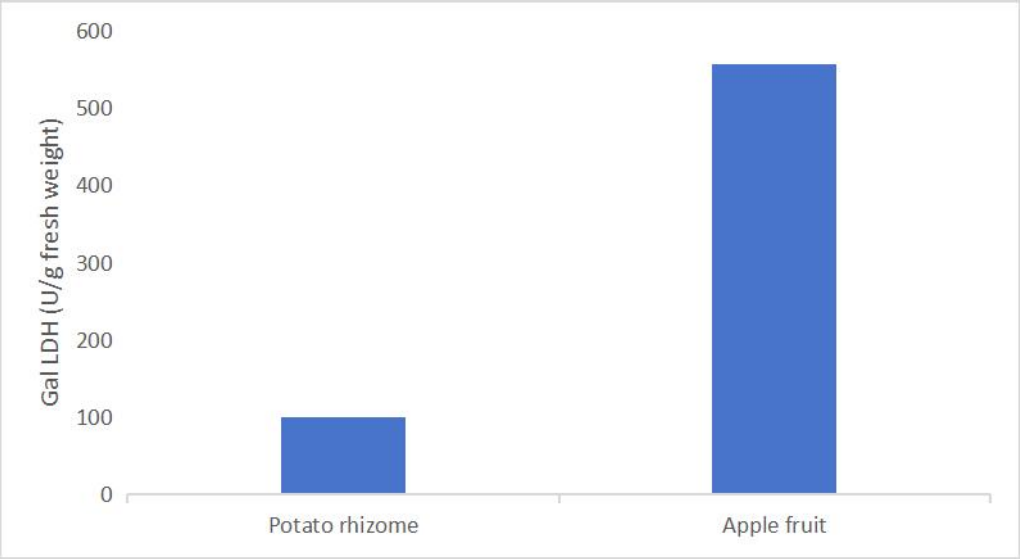

Figure 1. Determination Gal LDH activity in potato rhizome and Apple fruit by this assay kit.

Recommended Products

Catalog No.	Product Name
KTB3030	CheKine™ Micro Alcohol Dehydrogenase (ADH) Activity Assay Kit
KTB1560	CheKine™ Micro Alcohol Acyltransferase (AAT) Activity Assay Kit
KTB1270	CheKine™ Micro Pyruvate Dehydrogenase (PDH) Activity 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.