CheKine™ Micro Proline Dehydrogenase (ProDH) Activity Assay Kit

Cat #: KTB1431 Size: 48 T/96 T

	Micro Proline Dehydrogenase (ProDH) Activity Assay Kit		
REF	**Cat #:** KTB1431	LOT	**Lot #:** Refer to product label
	Applicable samples: Animal and Plant Tissues, Cells or Bacteria		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Proline dehydrogenase (ProDH) is a key enzyme that catalyzes the degradation of proline in mitochondria. Reducing the activity of ProDH is of great significance for regulating osmotic balance, preventing plant damage caused by osmotic stress, scavenging free radicals and protecting cell structure. CheKine™ Micro Proline Dehydrogenase (ProDH) Activity Assay Kit can be used to detect biological samples such as animal and plant tissues,cells or bacteria other liquid samples. In this kit, ProDH catalyzed the dehydrogenation of proline to pyruvic acid, and the removed hydrogen was transferred to reduce 2-dichlorophenol indophenol (DCPIP) by phenazine dimethyl ester sulfuric acid (PMS), and there was a characteristic absorption peak at 600 nm. Through the decrease of 600 nm absorbance, the reduction rate of 2-dichlorophenol indophenol (ProDH) was measured, which represented the activity of DCPIP.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	50 mL	100 mL	4℃
Reagent Ⅰ	1 mL	2 mL	4℃, protected from light
Reagent Ⅱ	12.5 mL	25 mL	4℃
Reagent Ⅲ	1	1	4℃, protected from light
Reagent Ⅳ	1	1	4℃, protected from light
Reagent Ⅴ	2	4	4℃, protected from light

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 420 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips, 1.5 mL EP tube
- Water bath, cryogenic centrifuge

- Deionized water, absolute ethanol
- Homogenizer or mortar (for tissue samples)

Reagent Preparation

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

Reagent I: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃, protected from light.

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

Reagent III: Prepared before use. Add 2 mL deionized water for 48 T and 4 mL deionized water for 96 T to fully dissolve. The prepared reagent can be stored at 4℃, protected from light for 6 month.

Reagent IV: Prepared before use. Add 2 mL deionized water for 48 T and 4 mL deionized water for 96 T to fully dissolve. The prepared reagent can be stored at 4℃, protected from light for 6 month.

Reagent V：Prepared before use. Add 0.5 mL deionized water to each bottle and fully dissolve it. This reagent is freshly prepared.

Working Reagent： Prepared before use. Mix well according to the proportion of Reagent II (V): Reagent III (V): Reagent IV (V)=2.4 (mL): 0.3 (mL): 0.3 (mL). Working Reagent is freshly prepared.

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. Tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize on ice. Centrifuge at 1,500 g for 15 min at 4℃. Take the supernatant into a new EP tube, add a drop of Reagent I (with a 10 μL precision pipette), mix well, place 30 min in an ice bath, and centrifuge 16,000 g at 4℃ for 20 min. Use supernatant for assay, and place it on ice to be tested.
2. Bacteria or Cells: Collect 5×10^6 bacteria or cells into the centrifuge tube, wash bacteria or cells with cold PBS, discard the supernatant after centrifugation; add 1 mL Extraction Buffer to ultrasonically disrupt the cells or bacteria 3 min (power 30% or 300 W, ultrasonic 3 s, interval 7 s). Centrifuge at 1,500 g for 15 min at 4℃.Take the supernatant into a new EP tube, add a drop of Reagent I (with a 10 μL precision pipette), mix well, place 30 min in an ice bath, and centrifuge 16,000 g at 4℃ for 20 min. Use supernatant for assay, and place it on ice to be tested.

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 420 nm. Visible spectrophotometer was returned to zero with deionized water.
2. Preheat Working Reagent at 30℃ for 10 min.
3. Sample measurement. (The following operations are operated in the 96-well plate or microglass cuvette)

Reagent	Test Well (μL)
Sample	35
Reagent V	15
Working Reagent	150

4. Mix thoroughly, detect the absorbance at 420 nm as A1 immediately and A2 after 10 min. Finally calculate ΔA=A2-A1.

Note: In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA is less than 0.1, increase the sample quantity appropriately. If ΔA is greater than 1.5, 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 the ProDH activity

(1) Calculated by sample protein concentration

Unit definition: The change of absorbance at 600 nm by 0.005 per milligram of tissue protein per minute per milliliter of reaction system is defined as a unit of enzyme activity.

ProDH (U/mg prot)=$\Delta A \times V_{Total} \div (V_{Sample} \times Cpr) \div 0.005 \div T$**=114.29×ΔA÷Cpr**

(2) Calculated by fresh weight of samples

Unit definition: The change of absorbance at 600 nm by 0.005 per gram of tissue per minute per milliliter of reaction system is defined as a unit of enzyme activity.

ProDH (U/g fresh weight)=$\Delta A \times V_{Total} \div (W \times V_{Sample} \div V_{Total\ sample}) \div 0.005 \div T$**=114.29×ΔA÷W**

(3) Calculated by bacteria or cells

Unit definition: The change of absorbance at 600 nm by 0.005 per 10^4 bacteria or cells per minute per milliliter of reaction system is defined as a unit of enzyme activity.

ProDH (U/10^4)=$\Delta A \times V_{Total} \div (n \times V_{Sample} \div V_{Total\ sample}) \div 0.005 \div T$**=114.29×ΔA÷n**

V_{Total}: total reaction volume, 0.2 mL; V_{Sample}: sample volume added, 0.035 mL; $V_{Total\ sample}$: the volume of adding Extraction Buffer, 1 mL; T: reaction time, 10 min; Cpr: Sample protein concentration, mg/mL; W: sample weight, g; n: total number of cells, in tens of thousands.

Typical Data

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

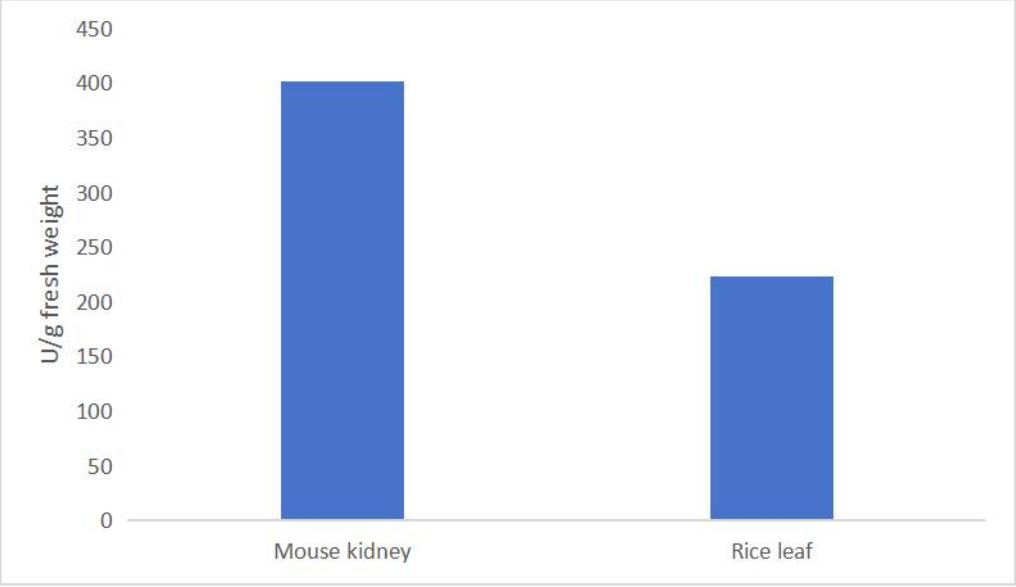

Figure 1. Determination of ProDH activity in mouse kidney and rice leaf by this kit.

Recommended Products

Catalog No.	Product Name
KTB1430	CheKine™ Micro Proline (PRO) Content Assay Kit

KTB1410	CheKine™ Micro Alanine Aminotransferase (ALT/GPT) Activity Assay Kit
KTB1420	CheKine™ Micro Aspartate Aminotransferase (AST/GOT) Activity Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.