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CheKine™ Micro Plant Dehydrogenase (PDHA) Activity Assay Kit

Cat #: KTB3023 Size: 48 T/96 T

	Micro Plant Dehydrogenase (PDHA) Activity Assay Kit		
REF	**Cat #:** KTB3023	LOT	**Lot #:** Refer to product label
	Applicable samples: Plant Tissues		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

The activity of plant dehydrogenase (PDHA) is largely reflects the active state of the organism, which can directly indicate the ability of biological cells to degrade its matrix. CheKine™ Micro Plant Dehydrogenase (PDHA) Activity Assay Kit can be used to detect biological samples such as plant tissues. In this kit, The hydrogen acceptor 2,3,5-triphenyl tetrazolium chloride (TTC) generates red triphenyl formazone (TFF) after receiving hydrogen during cell respiration. TFF has a characteristic absorption peak at 485 nm, the PDHA activity can quantified by measuring the absorbance at 485 nm.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Reagent Ⅰ	50 mL	100 mL	4℃, protected from light
Reagent Ⅱ	10 mL	20 mL	4℃

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 485 nm
- **96-well quartz plate / glass plate plate (non-polystyrene /polypropylene)** or microglass cuvette, precision pipettes, disposable pipette tips, 5 mL EP tube
- Water bath, cryogenic centrifuge
- Deionized water, ethyl acetate
- Homogenizer or mortar (for tissue samples)

Reagent Preparation

Reagent I: Prepared before use. Add 50 ml deionized water to each bottle to fully dissolve, Store at 4℃ for 1 week, protected from light.

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

Reagent Ⅲ: Ethyl acetate. **(Required but not provided)**

Sample Preparation

Plant tissues: Weigh 0.1 g fresh tissue (It is recommended to use colorless root samples), wash it with deionized water for 3-4 times, dry it with filter paper and set aside.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 485 nm. Visible spectrophotometer was returned to zero with **ethyl acetate**.
2. Sample measurement. (The following operations are operated in the 96-well plate or microglass cuvette)

Reagent	Test Well	Control Well
Sample (g)	0.1	0.1
Reagent Ⅰ (mL)	1	0
Reagent Ⅱ (mL)	1	2
Mix thoroughly and stand in dark for 3 hours at 37℃, ice bath for 5 min immediately after take out. Discard the filtrate, blot dry the sample with filter paper, place in homogenizer or mortar.		
Reagent Ⅲ (mL)	1	1

3. After fully grinding, fix the supplement volume to 2 mL with Reagent Ⅲ. Centrifuge at 12,000 g for 10 min at 4℃, take 200 μL of supernatant in a **96-well quartz plate / glass plate plate (non-polystyrene /polypropylene)** or micro glass cuvette to test the absorbance at 485 nm. The Test Well is marked as A_{Test}, and the Control Well is marked as $A_{control}$. Finally calculate $\Delta A_{Test}=A_{Test}-A_{control}$.

Note: In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If A is greater than 1.5 or ΔA is greater than 1, the supplement can be appropriately diluted with Reagent Ⅲ, 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 PDHA activity

Calculated by fresh weight of samples

Unit definition: One unit of enzyme activity is defined as the amount of 1 mg of tissue increases the absorbance of every 0.005 for per hour in the reaction system at 37℃.

PDHA (U/g fresh weight)=ΔA÷0.005÷T÷W**=66.7×ΔA÷W**

T: reaction time, 3 h; W: sample weight, g.

Precautions

1. After prepared, Reagent Ⅰ should store at 4℃, protect from light and used within one week. If it turns red, it cannot be used.
2. Immediately after the completion of the reaction, take an ice bath to terminate the reaction, and remove the residual reaction solution as clean as possible.
3. Reagent Ⅲ is volatile, toxic, Reagent Ⅲ-related reactions, please wear a lab coat, mask, latex gloves, operate in the ventilation cupboard.
4. **It is suggested that fresh colorless root samples be used to detect PDHA. For colored samples, before adding Reagent Ⅲ, we can grind the supernatant with deionized water, centrifuge at 12,000 g for 10 min at 4℃, discard the**

supernatant, precipitate and grind with Reagent III, collect the grinding solution and fix the volume, follow Assay Procedure and centrifuge the supernatant for detection.

Typical Data

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

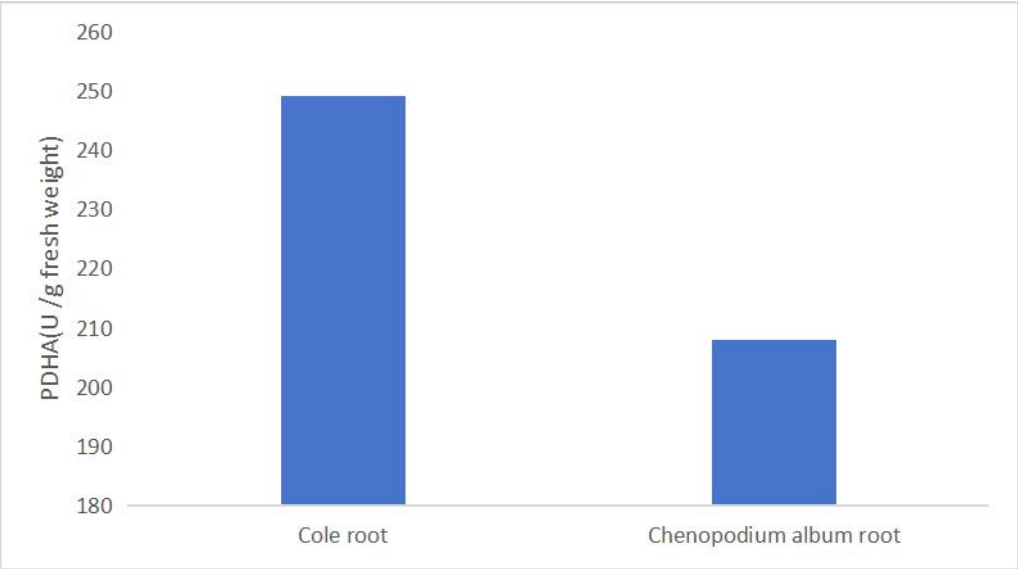

Figure 1. Determination of PDHA activity in cole root and chenopodium album root by this kit.

Recommended Products

Catalog No.	Product Name
KTB1127	CheKine™ Micro Acetokinase (ACK) Activity Assay Kit
KTB1023	CheKine™ Micro Citrate Synthase (CS) Activity Assay Kit

Disclaimer

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