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CheKine™ Micro Plant Root Vitality (TTC-Method) Assay Kit

Cat #: KTB3025 Size: 48 T/96 T

	Micro Plant Root Vitality (TTC-Method) Assay Kit		
REF	**Cat #:** KTB3025	LOT	**Lot #:** Refer to product label
	Applicable samples: Plant Root 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 Root Vitality (TTC-Method) 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 A	1	1×2	4℃, protected from light
Reagent Ⅰ B	1	1×2	4℃
Reagent Ⅱ	100 mL	100 mL×2	4℃
Reagent Ⅲ	1×2	1×4	4℃
Standard	1	1	4℃, protected from light

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 485 nm
- **96-well quartz plate / glass 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 A: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃, protected from light.

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

Reagent Ⅰ: Prepared before use. Add one Reagent Ⅰ B and 50 ml deionized water to Reagent Ⅰ A 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: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃.

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

Standard: Prepared before use. Add 1 ml deionized water to fully dissolve, that is 10 mg/mL TTC Standard. Store at 4℃ for 1 week, protected from light.

20 μg/mL Standard: Take 10 μL 10 mg/mL TTC Standard, add 1,990 μL deionized water and mix well to make 50 μg/mL TTC Standard. Take 1 mL 50 μg/mL TTC Standard and add it into a Reagent Ⅲ, fully shake and mix for 2 min. After mixing, add 1 mL of Reagent Ⅳ, fully shake and mix again for 2 min, stand at room temperature for stratification for 5 min, and take the upper solution, that is, 50 μg/mL TTC Standard. Absorb 200 μL, 50 μg/mL TTC Standard, add 300 μL Reagent Ⅳ, and mix well to 20 μg/mL TTC Standard. 20 μg/mL TTC Standard is freshly prepared. The 20 μg/mL TTC Standard was used for follow-up test.

Note: After prepared, Reagent Ⅰ and Standard should store at 4℃, protect from light and used within one week. If it turns red, it cannot be used.

Sample Preparation

Note: Fresh root samples are used to detect plant root vitality, and the samples should not be frozen for use.

Plant root tissues: Weigh 0.1 g fresh tissue (It is recommended to use colorless root samples), rinse 3-4 times with deionized water, dry gently, do not oversqueeze and destroy root cells.

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. Determination of Standard:

Take 200 μL 20 μg/mL TTC Standard and 200 μL Reagent Ⅳ in a **96-well quartz plate / glass plate (non-polystyrene /polypropylene)** or microglass cuvette to test the absorbance at 485 nm. The Standard Well is marked as $A_{Standard}$, and the Reagent Ⅳ Well is marked as A_{Blank}. Finally calculate $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.

3. Sample measurement. (The following operations are operated in the 5 mL EP tube)

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 4 h 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

4. After fully grinding, centrifuge at 12,000 g for 10 min at 4℃, take 200 μL of supernatant in a **96-well quartz plate / glass plate (non-polystyrene /polypropylene)** or microglass 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: (1) The Blank Well and the Standard 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 A_{Test} is greater than 1.5 or ΔA_{Test} is greater than 1, the supplement can be appropriately diluted with Reagent Ⅳ, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately. (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 Ⅳ is volatile, toxic, Reagent Ⅳ-related reactions, please wear a lab coat, mask, latex gloves, operate in the ventilation cupboard. (4) It is recommended that fresh colorless root samples be used to detect plant root vitality. For colored samples, before adding Reagent Ⅳ, 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 Ⅳ, collect the grinding solution and fix the volume, follow Assay Procedure and centrifuge the supernatant for detection.

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 plant root vitality

Calculated by fresh weight of samples

Unit definition: The root activity was expressed by the reduction amount of TTC.

Reduction strength of TTC [μg TTC/(g·h)]=$\Delta A_{Test} \times C_{Standard} \div \Delta A_{Standard} \times V \div (W \times T)$**=$5 \times \Delta A_{Test} \div \Delta A_{Standard} \div W$**

$C_{Standard}$: Standard concentration, 20 μg/mL; W: weight of root, g; T: Reaction time, 4 h; V: The volume of Reagent Ⅳ, 1 mL.

Typical Data

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

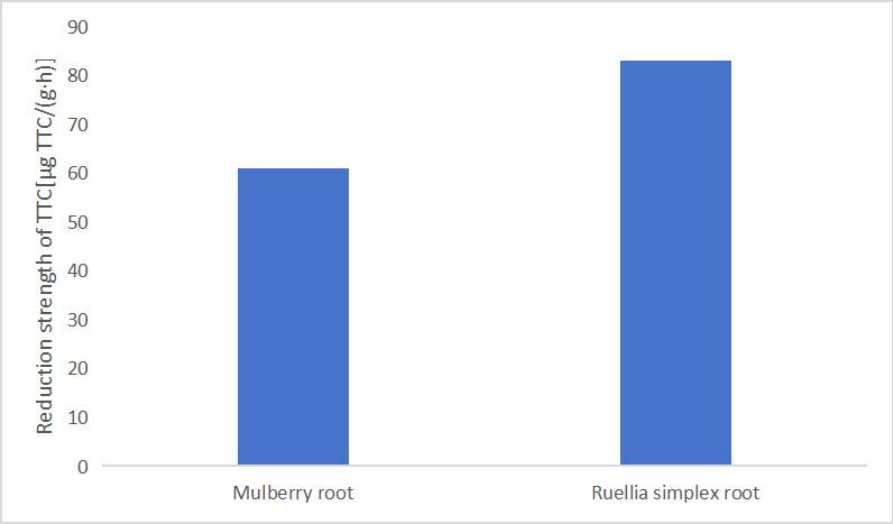

Figure 1. Determination of plant root vitality in mulberry root and ruellia simplex root by this kit.

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.