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CheKine™ Micro Chalcone Isomerase (CHI) Activity Assay Kit

Cat #: KTB2401

Size: 48 T/96 T

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REF	Cat # : KTB2401	LOT Lot #: Refer to product label	
	Applicable sample: Plant tissues		
X	Storage: Stored at 4°C for 6 months, protected from light		

Assay Principle

Chalcone isomerase (CHI) is the first recognized enzyme related to flavonoid synthesis and one of the key enzymes in flavonoid metabolism. Chalcone isomerase and chalcone synthase together constitute rate-limiting enzymes for flavonoid biosynthesis. CheKine™ Micro Chalcone Isomerase (CHI) Activity Assay Kit can detect plant tissues samples. In this kit, CHI catalyzed the cyclization of chalcone to form 4,5,7-trihydroxyflavanones, and the activity of CHI was indicated by measuring the absorbance change at 381 nm.

Materials Supplied and Storage Conditions

Kit componente	Size		Storage conditions
Kit components	48 T	96 T	Storage conditions
Extraction Buffer	50 mL	100 mL	4°C, protected from light
Reagent I	12.5 mL	25 mL	4°C
Reagent II	1	1	4°C, protected from light

Materials Required but Not Supplied

- Microplate reader or ultraviolet spectrophotometer capable of measuring absorbance at 381 nm
- 96-well microplate or microquartz cuvette, precision pipettes, disposable pipette tips
- Water bath, cryogenic centrifuge,1.5 mL EP tube
- Deionized water
- Mortar or homogenizer (for tissue samples)

Reagent Preparation

Extraction Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C, protected from light. Note: The Extraction Buffer has a pungent odor, so it is recommended to experiment in a fume hood.



Reagent I: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

Reagent II: Prepared before use. Add 10 mL Reagent | for 48 T and 20 mL Reagent | for 96 T to fully dissolve. The remaining reagent can also be stored at 4°C and protected from light for 1 month.

Sample Preparation

Note: We recommend that you use fresh samples. If not assayed immediately, samples can be stored at -80°C 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 0.1 g tissue, add 1 mL Extraction Buffer and homogenize on ice. Centrifuge at 8,000 g for 10 min at 4°C. Use supernatant for assay, and place it on ice to be tested.

Note: It will be better to quantify the total protein with Protein Quantification Kit (Bradford Assay), Cat #: KTD3002, if it is calculated by protein concentration.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 381 nm, ultraviolet spectrophotometer was returned to zero with deionized water.

2. Operation table (The following operations are operated in the 96-well UV microplate or microquartz cuvette):

Reagent	Test Well (μL)
Sample	10
Reagent II	190

3. Mix thoroughly, measure the absorbance value A_1 at 10 s at 381 nm, and the absorbance value A_2 at 30 min 10 s at 37°C for 30 min. Finally calculate $\Delta A = A_2 - A_1$.

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 0.05, 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 CHI activity:

(1) Calculated by protein concentration

Active unit definition: A change of 0.05 per hour in A381 per milligram of protein per milliliter of the reaction system is defined as a unit of enzyme activity.

CHI (U/mg prot)=ΔA×V_{Total}÷(V_{Sample}×Cpr)÷T÷0.05=800×ΔA÷Cpr

(2) Calculated by fresh weight of samples

Active unit definition: A change of 0.05 per hour in A381 per gram tissue per milliliter of the reaction system is defined as one unit of enzyme activity.

CHI (U/g fresh weight)=ΔA×V_{Total}÷(W×V_{Sample}÷V_{Total sample})÷T÷0.05**=800×ΔA÷W**

V_{Total}: total reaction volume, 0.2 mL; V_{Sample}: sample volume added, 0.01 mL; V_{Total sample}: Extraction Buffer volume added, 1 mL; T: reaction time, 0.5 h; Cpr: sample protein concentration, mg/mL; W: weight of sample, g.

Typical Data

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







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.

