CheKine™ Micro Neutral Invertase (NI) Activity Assay Kit

Cat #: KTB2291 Size: 48 T/96 T

	Micro Neutral Invertase (NI) Activity Assay Kit		
REF	**Cat #:** KTB2291	LOT	**Lot #:** Refer to product label
	Applicable sample: Plant tissues		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Invertase (Ivr) catalyzes the irreversible decomposition of sucrose into fructose and glucose, and is one of the key enzymes in sucrose metabolism in higher plants. According to the optimal pH, higher plant Ivr can be divided into acidic invertase (AI) and Neutralinvertase (NI) types. CheKine™ Micro Neutral Invertase (NI) Activity Assay Kit can detect plant tissues samples. In this kit, NI catalyzes sucrose decomposition to produce reducing sugar, which further reacts with 3,5-dinitrosalicylic acid to produce brown-red amino compounds, which have characteristic light absorption at 540 nm, and the light absorption value at 540 nm is proportional to the amount of reducing sugar generation in a certain range. NI activity was calculated by increasing rate of light absorption.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	50 mL	100 mL	4℃
Reagent Ⅰ	10 mL	20 mL	4℃
Reagent Ⅱ	1	1	4℃
Reagent Ⅲ	7.5 mL	15 mL	4℃, protected from light
Standard	1	1	4℃, protected from light

Materials Required but Not Supplied

- Microplate reader or ultraviolet spectrophotometer capable of measuring absorbance at 540 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℃.

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℃.

Reagent Ⅱ: Prepared before use. Add 5 mL Reagent Ⅰ for 48 T and 10 mL Reagent Ⅰ for 96 T to fully dissolve. The remaining reagent can also be stored at 4℃ for 2 weeks.

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

Standard: Prepared before use. Add 1 mL deionized water and fully dissolve to 10 mg/mL. The remaining reagent can also be stored at 4℃ for 1 month. Use the 10 mg/mL standard solution and further dilute it to the standard as shown in the following table:

Num.	Standard Volume (μL)	Deionized Water (μL)	Concentration (mg/mL)
Std.1	200 μL 10 mg/mL Standard	800	2
Std.2	600 μL of Std.1 (2 mg/mL Standard)	200	1.5
Std.3	200 μL of Std.1 (2 mg/mL Standard)	200	1
Std.4	200 μL of Std.3 (1 mg/mL Standard)	200	0.5
Std.5	200 μL of Std.4 (0.5 mg/mL Standard)	200	0.25
Blank	0	400	0

Notes: Always prepare fresh Standards per use; Diluted Std. solution is unstable and must be used within 4 h.

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 0.1 g tissue, add 1 mL Extraction Buffer and homogenize on ice. Centrifuge at 12,000 g for 10 min at 4℃. 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 540 nm, ultraviolet spectrophotometer was returned to zero with deionized water.

2. Operation table (The following operations are operated in the 1.5 mL EP tube):

Reagent	Blank Well (μL)	Standard Well (μL)	Control Well (μL)	Test Well (μL)
Sample	0	0	50	50
Standard	0	50	0	0
Deionized water	50	0	0	0
Reagent Ⅰ	0	0	200	0
Reagent Ⅱ	200	200	0	200

Mix well, hold at 37℃ for 30 min, place in 95℃ water bath for 10 min (cover tightly to prevent water loss), and cool down with running water.

Reagent III	125	125	125	125

3. Mix well, bathe in water at 95℃ for 10 min (cover tightly to prevent water loss), cool down with running water and mix well, take 200 μL into 96-well microplate or microquartz cuvette, and record the absorbance value at 540 nm. The Blank Well is recorded as A_{Blank}, the Standard Well is marked as $A_{Standard}$, the Control Well is marked as $A_{Control}$, and the Test Well is marked as A_{Test}. Finally calculate $\Delta A_{Test}=A_{Test}-A_{Control}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.

Note: 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 ΔA is greater than 1.6, 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.

1. Drawing of standard curve

With the concentration of the standard solution as the x-axis and the $\Delta A_{Standard}$ as the y-axis, draw the standard curve and obtain the standard equation y=kx+b. The determination of ΔA_{Test} is brought into the equation to get x (mg/mL).

2. Calculation of the NI activity

(1) Calculated by protein concentration

Active unit definition: The production of 1 μg reducing sugar per milligram of protein per min at 37℃ was defined as one unit of enzyme activity.

NI (U/mg prot)=$x\times V_{Sample}\div(V_{Sample}\times Cpr)\div T\times 1{,}000$**=33.3x÷Cpr**

(2) Calculated by fresh weight of samples

Active unit definition: The production of 1 μg reducing sugar per gram tissue per min at 37℃ was defined as one unit of enzyme activity.

NI (μg/g fresh weight)=$x\times V_{Sample}\div(V_{Sample}\times W\div V_{Total\ sample})\div T\times 1{,}000$**=33.3x÷W**

V_{Sample}: sample volume added, 0.05 mL; $V_{Total\ sample}$: Extraction Buffer volume added, 1 mL; T: reaction time, 30 min; Cpr: sample protein concentration, mg/mL; W: weight of sample, g; 1,000: conversion factor, 1 mg/mL=1,000 μg/mL.

Precautions

1. If Reagent III is added and turbidity appears after 10 min of water bath at 95℃, it is recommended to centrifuge at 4℃ for 5 min at 12,000 g and then take supernatant to measure absorbance.
2. Since the Extraction Buffer contains a certain concentration of protein (about 1 mg/mL), it is necessary to subtract the protein content of the extraction solution itself when determining the protein concentration of the sample.

Typical Data

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

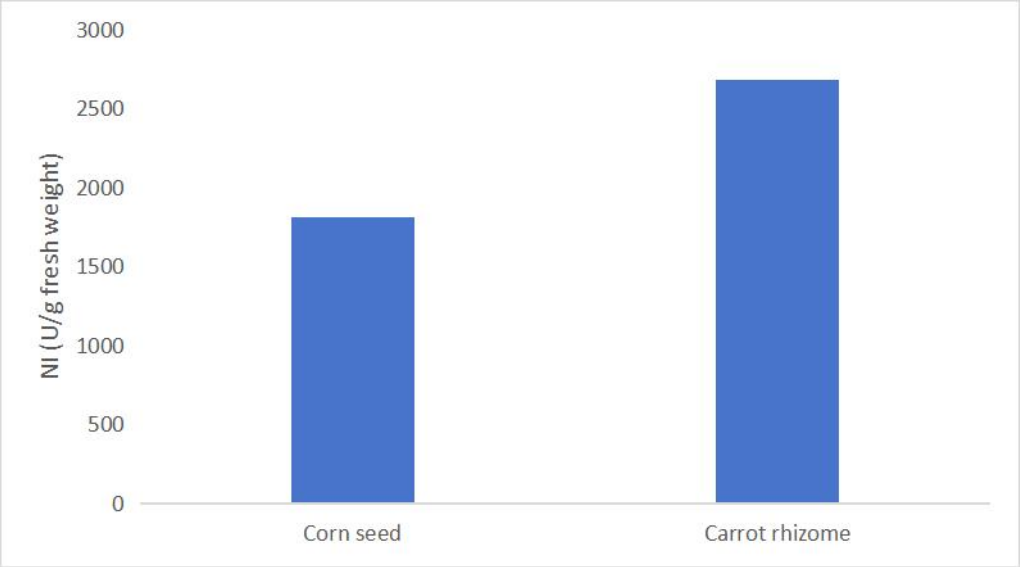

Figure 1. Determination NI activity in corn seed and carrot rhizome by this assay kit.

Recommended Products

Catalog No.	Product Name
KTB3110	CheKine™ Micro Sucrose Synthetase (SS) 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.