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CheKine™ Micro Soil Available Boron Content Assay Kit

Cat #: KTB4051 Size: 48 T/48 S 96 T/96 S

	Micro Soil Available Boron Content Assay Kit		
REF	**Cat #:** KTB4051	LOT	**Lot #:** Refer to product label
	Applicable sample: Soil sample		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

The available boron in the soil directly affects the plant's absorption and utilization. CheKine™ Micro Soil Available Boron Content Assay Kit provides a simple, convenient, and rapid method for detecting available boron content in soil samples. The principle of this assay is that boron forms a yellowish-brown complex with azomethine in weakly acidic conditions, which has a characteristic absorption peak at 420 nm.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Reagent Ⅰ	3 mL	6 mL	4℃
Reagent Ⅱ	Powder×1 vial	Powder×2 vials	4℃
Reagent Ⅲ	Powder×1 vial	Powder×2 vials	4℃, protected from light
Standard	Powder×1 vial	Powder×1 vial	4℃

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

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
- Oven, 100 mesh sieve, centrifuge, constant temperature water bath, analytical balance
- Deionized water

Reagent Preparation

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

Working Reagent Ⅱ: Ready to use as supplied. Take one vial and dissolve it in 10 mL of deionized water and add 156.25 μL of acetic acid; then mix thoroughly and set aside for use. Any unused reagent can be stored at 4℃ for up to one week.

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Working Reagent III: Ready to use as supplied. Take one vial and dissolve in 5 mL of deionized water; Any unused reagent can be stored at 4℃ in the dark for up to one week.

Standard: Ready to use as supplied. Dissolve by adding 0.81 mL of deionized water to prepare a 200 μmol/mL standard solution for use. Any unused reagent can be stored at 4℃ in the dark for up to two weeks.

Note: Reagent III is toxic, so it is recommended to experiment in a fume hood.

Standard preparation: Using a 200 μmol/mL standard solution, dilute it 10-fold with deionized water to prepare a 20 μmol/mL standard solution. Then, further dilute it according to the table below to create the standards:

Num.	Standard Volume	Deionized Water Volume (μL)	Concentration (μmol/mL)
Std.1	75 μL 20 μmol/mL Standard	925	1.5
Std.2	400 μL of Std.1 (1.5 μmol/mL)	200	1
Std.3	200 μL of Std.2 (1 μmol/mL)	200	0.5
Std.4	200 μL of Std.3 (0.5 μmol/mL)	200	0.25
Std.5	200 μL of Std.4 (0.25 μmol/mL)	200	0.125
Std.6	200 μL of Std.5 (0.125 μmol/mL)	200	0.0625
Blank	0	500	0（Blank Tube）

Notes: Always prepare fresh standards per use; Diluted Standard Solution is unstable and must be used within 4 h.

Sample Preparation

Note: Fresh samples are recommended.

Air-dry fresh soil samples naturally or in a 37℃ oven, then sieve through a 100-mesh sieve. Weigh approximately 0.5 g of the sieved soil sample into a 2 mL capped centrifuge tube, add 1 mL of deionized water, and extract by placing the tube in boiling water for 10 minutes. Allow the sample to cool to room temperature, then add 50 μL of Reagent I, shake for 5 min, and centrifuge at 8,000 g for 10 min at 25℃. Collect the supernatant for further testing.

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. Operation table (The following operations are to be carried out in a 96-well plate or 1.5 mL EP tube):

A. Steps for Determining the Absorbance of the Supernatant from Colored Samples

Reagent	Test Well (μL)	Control Well (μL)	Standard Well (μL)
Supernatant	40	40	0
Standard	0	0	40
Reagent II	80	80	80
Reagent III	40	0	40
Deionized water	40	80	40

Mix thoroughly and keep in the dark at 25℃ for 1 h. Measure the absorbance at 420 nm using a 96-well plate or a micro glass cuvette. Record the absorbance values as A_{Test}, $A_{Control}$, $A_{Standard}$, and A_{Blank}, respectively. Calculate $\Delta A_{Test}=A_{Test}-A_{Control}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.

B. Steps for Determining the Absorbance of the Supernatant from Colorless Samples:

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Reagent	Test Well (μL)	Standard Well (μL)
Supernatant	40	0
Standard	0	40
Reagent II	80	80
Reagent III	40	40
Deionized water	40	40
Mix thoroughly and keep in the dark at 25℃ for 1 h. Measure the absorbance at 420 nm using a 96-well plate or a micro glass cuvette. Record the absorbance values as A_{Test}, $A_{Standard}$, and A_{Blank}, respectively. Calculate $\Delta A_{Test}=A_{Test}-A_{Blank}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.		

Note:The standard curve needs to be determined only once. Before the experiment, it is suggested that 2-3 samples with large expected differences should be selected for pre-experiment. If ΔA_{Test} is less than 0.03, the sample volume can be appropriately increased, and the calculation formula should be adjusted accordingly. If ΔA_{Test} is greater than 1.2, the sample supernatant can be further diluted by deionized water, and the calculation result should be multiplied by the dilution multiple.

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, get the standard equation, and bring the ΔA_{Test} into the equation to get the x value (μmol/mL).

2. Calculation of available boron content:

Available boron content (mg/kg soil sample)=$10.81\times x\times V_{Total}\div 1{,}000\div(W\div 1{,}000)$**$=11.35\times x\div W$**

Where: V_{Total}: Total sample volume, 1.05 mL; 10.81: Molecular weight of boron, 10.81 μg/μmol; 1,000: Conversion factor, 1 mmol=1,000 μmol, 1 kg=1,000 g; W: Sample mass, g.

Typical Data

Typical standard curve:

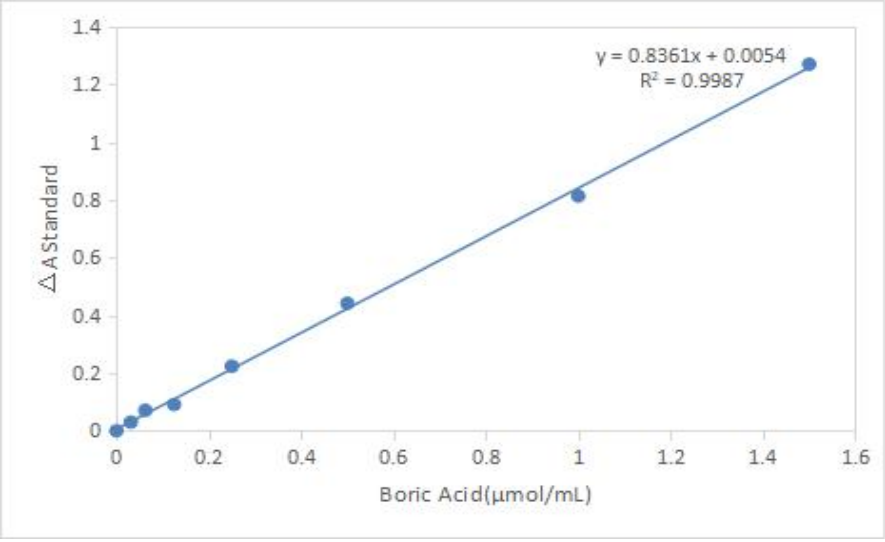

Figure1. Boric acid standard curve

Examples:

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Take 0.5 g of fresh soil sample that has been dried in a 37℃ oven and use 96-well plate to calculate ΔA_{Test}=0.17-0.15=0.02, x=0.017. The content calculated according to the soil sample mass is as follows:

Available boron content (mg/kg soil sample)=11.35×0.017÷0.5=0.3859 mg/kg soil sample.

Recommended Products

Catalog No.	Product Name
KTB4023	CheKine™ Mirco Soil Peroxidase (S-POD) Activity Assay Kit
KTB4024	CheKine™ Mirco Soil Acid Protease (S-ACPT) Activity Assay Kit
KTB4025	CheKine™ Mirco Soil β-Xylosidase (S-β-XYS) Activity Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.

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