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CheKine™ Micro Soil Total Iron Content Assay Kit

Cat #: KTB4032 Size: 48 T/48 S 96 T/96 S

	Micro Soil Total Iron Content Assay Kit		
REF	**Cat #:** KTB4032	LOT	**Lot #:** Refer to product label
	Detection range: 62.5-4000 mg/kg		**Sensitivity:** 62.5 mg/kg
	Applicable sample: Soli		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Iron is a very important plant nutrient element, and the iron content in soil directly affects the absorption and utilization of plants and their growth and metabolism. CheKine™ Micro Soil Total Iron Content Assay Kit can detect biological samples such as soli. In this kit, in the range of pH 2-9, hydroxylamine hydrochloride converts trivalent iron into divalent iron, which reacts with o-phenanthroline to form an orange-red complex with a characteristic absorption peak at 510 nm. By detecting the absorbance change at 510 nm, the total iron content in soil can be calculated.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Powder	Powder×1 vial (25 g)	Powder×1 vial (50 g)	RT
Reagent Ⅰ	Powder×1 vial	Powder×2 vials	4℃, protected from light
Reagent Ⅱ	4.2 mL	8.4 mL	4℃
Reagent Ⅲ	2.8 mL	5.6 mL	4℃, protected from light
Standard	Powder×1 vial	Powder×1 vial	4℃, protected from light

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 510 nm
- 96-well microplate or microglass cuvette, precision pipettes, disposable pipette tips,1.5 mL EP tube
- Water bath, freezing centrifuge, Muffle furnace, crucible, crucible clamp, 100 mesh sieve
- Deionized water, hydrochloric acid

Reagent Preparation

Extraction Powder: Ready to use as supplied. Store at room temperature.

Extraction Buffer: Prepared before use. According to the dosage, concentrated hydrochloric acid and deionized water are prepared in a ratio of 1: 1.**(Required but not provided)**

Working Reagent I: Prepared before use. Add 1.4 mL deionized water to each bottle, dissolve thoroughly. The remaining reagent can be stored at 4℃, protected from light for 1 week.

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℃, protected from light.

Note: Extraction Buffer or Reagent III has certain irritation, so personal protection is recommended during use.

Standard: Prepared before use. Add 1 mL deionized water to a bottle, dissolve thoroughly, that is 1,000 mg/L iron Standard. The remaining reagent can be stored at 4℃ ,protected from light for 1 month. Using 1,000 mg/L iron Standard solution, prepare standard curve dilution as described in the table:

Num.	Standard Volume (μL)	Deionized Water (μL)	Concentration (mg/L)
Std.1	20 μL of 1,000 mg/L Standard	180	100
Std.2	100 μL of Std.1 (100 mg/L)	100	50
Std.3	100 μL of Std.2 (50 mg/L)	100	25
Std.4	100 μL of Std.3 (25 mg/L)	100	12.5
Std.5	100 μL of Std.4 (12.5 mg/L)	100	6.25
Std.6	100 μL of Std.5 (6.25 mg/L)	100	3.125
Std.7	100 μL of Std.6 (3.125 mg/L)	100	1.5625
Blank	0	100	0

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

Sample Preparation

Note: Note: It is recommended to use fresh soil samples.

Fresh soil samples naturally air dried or air dried in an oven at 37℃ and sieved through 100 mesh sieve. According to the ratio of soil mass (g): Extraction Powder mass (g) of 1: 4 (it is recommended to weigh about 0.1 g soil sample and add 0.4 g Extraction Powder), slowly add the Extraction Powder into the crucible, and stir evenly while adding; Then melting in a muffle furnace at 550℃ for 10 min; Then melting at 920℃ for 30 min; Take out the crucible while it is hot, transfer the melt into a beaker, add 4 mL of Extraction Buffer while stirring, cover it if necessary to prevent the solution from splashing, dissolve it for 30 min, centrifuge at 5,000 g, 25℃ for 10 min, and take the supernatant to be measured.

Note: If the molten material adheres to the crucible and cannot be poured out, the molten material can be dissolved by adding a small amount of Extraction Buffer to the crucible several times, and the total volume of Extraction Buffer needs to be 4 mL. The temperature of the molten material is very high, so be careful to prevent scalding.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 510 nm, visible spectrophotometer was returned to zero with deionized water.

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

Reagent	Blank Well (μL)	Standard Well (μL)	Test Well (μL)

Sample	0	0	20
Standard	0	20	0
Deionized Water	80	60	60
Working Reagent Ⅰ	20	20	20
Reagent Ⅱ	60	60	60
Reagent Ⅲ	40	40	40

Fully mixing, standing at 25℃ for 20 min, record the absorbance value at 510 nm. The Blank Well is recorded as A_{Blank}, the Standard Well is marked as $A_{Standard}$, the Test Well is marked as $A_{Test.}$ Finally calculate $\Delta A_{Test}=A_{Test}-A_{Blank}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.

Note: The Standard Well and Blank Well only need to be done once or twice. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA_{Test} is less than 0.01, increase the sample quantity appropriately. If ΔA_{Test} is larger than 100 mg/L of $\Delta A_{Standard}$, 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. The determination of ΔA_{Test} is brought into the equation to get x (mg/L).

2. Calculation of the total iron content

Total iron (mg/kg soli)=$x\times V_{Total\ sample}\div w$**$=4x\div w$**

$V_{Total\ sample}$: total reaction volume, 4 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.

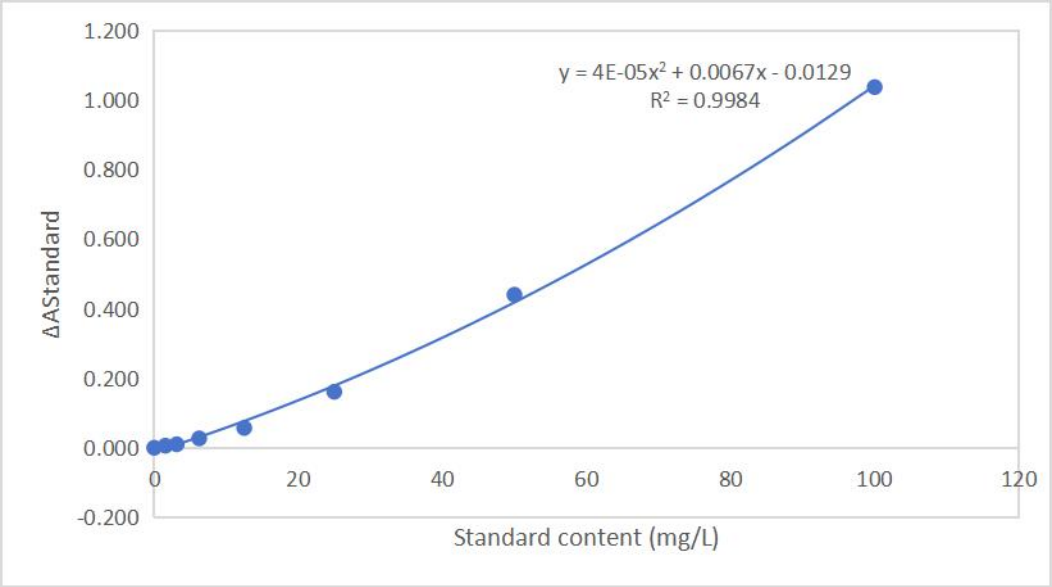

Figure 1. Standard curve of total iron.

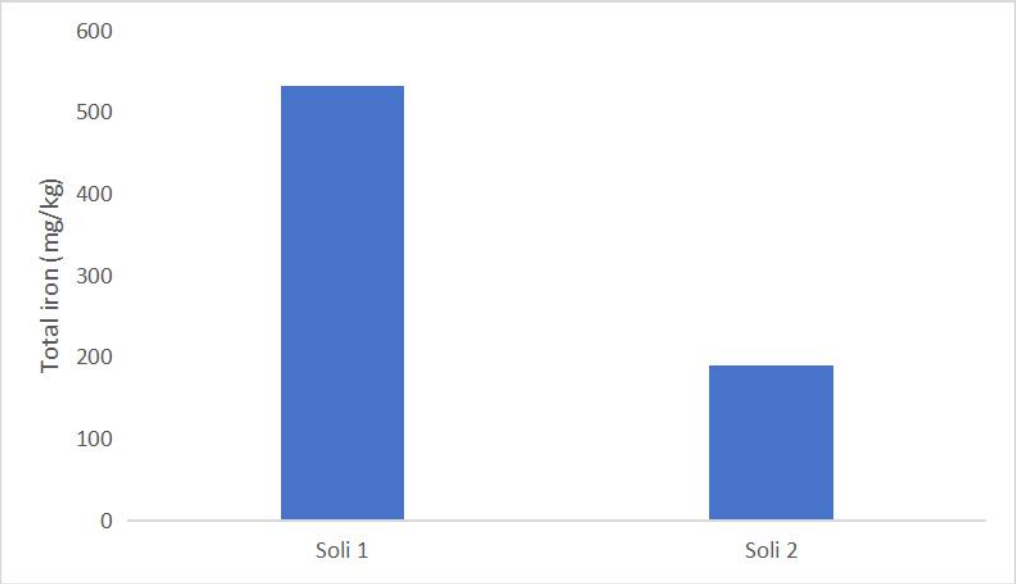

Figure 2. Determination total iron content in soli sample by this assay kit.

Recommended Products

Catalog No.	Product Name
KTB4012	CheKine™ Micro Soil Nitrate Nitrogen Assay Kit
KTB4014	CheKine™ Micro Acid Soil Available Phosphorous Assay Kit
KTB4041	CheKine™ Micro Soil Alkaline Phosphatase(S-AKP/ALP) Activity Assay Kit
KTB4050	CheKine™ Micro Soil Catalase (S-CAT) 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.