

## Soil Total Iron Content Assay Kit

**Note:** Take two or three different samples for prediction before test.

**Operation Equipment:** Microplate reader/Spectrophotometer

**Catalog Number:** BC3005

**Size:** 100T/96S

**Product Composition:** Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact Solarbio staff in time.

Reagent Name	Size	Preservation Condition
Extraction agent	Powder×1	2-8°C
Extract solution	Liquid 120 mL×2	2-8°C
Reagent I	Liquid 3 mL×1	2-8°C
Reagent II	Liquid 8 mL×1	2-8°C
Reagent III	Liquid 5 mL×1	2-8°C

### Product Description:

Iron is a very important plant nutrient, and the content of iron in the soil directly affects the absorption and utilization by plants as well as their growth and metabolism. Within the pH range of 2-9, hydroxylamine hydrochloride converts ferric iron ( $\text{Fe}^{3+}$ ) to ferrous iron ( $\text{Fe}^{2+}$ ), which then reacts with o-phenanthroline to form an orange-red complex that has a characteristic absorption peak at 510nm. By measuring the change in absorbance at 510nm, the total iron content in the soil can be calculated.

### Reagents and Equipment Required but Not Provided:

Visible Spectrophotometer/Microplate reader, balance, muffle furnace, crucible, crucible tongs, benchtop centrifuge, water bath, micro quartz cuvette/96 well plate, adjustable pipette, 100-mesh sieve, and distilled water.

### Operation procedure:

**I. Sample preparation**(The sample size to be tested can be adjusted appropriately, and the specific proportion can be referred to the literature.)

1. Take fresh soil samples and dry them, then pass them through a 100-mesh sieve.
2. According to the ratio of soil mass (g) to extraction agent mass (g) of 1:4 (it is recommended to weigh about 0.05g of soil and add 0.2g of extraction agent), slowly add the extraction agent to the crucible while stirring evenly.
3. Then fuse in a muffle furnace at 550°C for 10 minutes; then fuse at 920°C for 30 minutes.
4. Remove the crucible while it is still hot, transfer the fused material to a beaker, and add 2mL of extraction solution while stirring, cover if necessary to prevent the solution from splashing out, and dissolve for 30 minutes.
5. Finally, centrifuge at 5000g at 25°C for 10 minutes, and take the supernatant for testing.

## II. Determination procedure:

1. Preheat visible spectrophotometer/microplate for 30 minutes, adjust the wavelength to 510 nm, set the counter to zero with distilled water.
2. Add the following reagents to 1.5mLEP tube/96 well plate

Reagent name (μL)	Test tube (A <sub>T</sub> )	Blank tube (A <sub>B</sub> )
Sample	20	-
Reagent I	20	20
Reagent II	60	60
Reagent III	40	40
Distilled water.	60	80

Thoroughly mix the solution, let it stand at 25°C for 20 minutes to allow the reaction to occur.

Transfer the reaction mixture to micro glass cuvettes/96 well plates and measure the absorbance at 510nm, recording it as A<sub>T</sub> and A<sub>B</sub>. Calculate  $\Delta A_T = A_T - A_B$ . The blank tube only needs to be measured 1-2 times.

## III. Calculation:

### 1. Calculation formula of determination with micro glass cuvette

Standard Curve:  $y = 0.1569x - 0.0173$ ,  $R^2 = 0.9992$

Total Iron Content (mg/kg) =  $(\Delta A_T + 0.1073) \div 0.1569 \times V_s \div W = 25.494 \times (\Delta A_T + 0.1073) \div W$

V<sub>s</sub>: The volume of extraction solution added is 4 mL;

W: Sample weight(g)

### 2. Calculation formula of determination with 96 well plate

Standard Curve:  $y = 0.0785x - 0.0173$ ,  $R^2 = 0.9992$

Total Iron Content (mg/kg) =  $(\Delta A_T + 0.1073) \div 0.0785 \times V_s \div W = 50.956 \times (\Delta A_T + 0.1073) \div W$

V<sub>s</sub>: The volume of extraction solution added is 4 mL;

W: Sample weight(g)

### Note:

1. If the  $\Delta A_T > 1.5$  or the  $\Delta A_T > 1.0$ , it is recommended to dilute the sample with the extraction solution before testing. If the measured absorbance is too low or close to the blank value, it is recommended that the client increases the sample amount and retests, making sure to adjust the calculation formula accordingly.