Cell Iron Content Assay Kit

Note: It is necessary to predict 2-3 large difference samples before the formal determination.

Operation Equipment: Spectrophotometer/microplate reader

Cat No: BC5315

Size: 100T/96S

Components:

Extract solution: Liquid 60 mL×1. Store at 2-8℃.

Reagent I: Liquid 5 mL×1. Store at 2-8℃.

Reagent II: Liquid 15 mL×1. Store at 2-8℃.

Reagent III: Liquid 2.5 mL×1. Store at 2-8℃.

Standard: Liquid 1 mL×1. Store at 2-8℃. 1 μmol/mL of Fe^{3+} standard solution. Add 100μL distilled water to 100μL Fe^{3+} standard solution of 1 μmol/mL before use, and mix well to form the Fe^{3+} standard solution of 0.5 μmol/mL.

Product Description:

Iron is one of the essential trace elements in human body, which is the main component of hemoglobin, myoglobin, cytochrome and other enzyme systems. Iron can assist in the transport of oxygen and promote fat oxidation. Iron deficiency can easily cause anemia, metabolic disorders, and affect the immune function of the body.

Fe^{3+} is reduced by hydroxylamine hydrochloride to Fe^{2+}. Fe^{2+} could react with Phenanthroline Monohydrate to form a kind of orange compound under weak acid condition, which has an absorption peak at 510 nm. According measure absorbance at 520 nm can reflect cell iron concentration.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/Microplate reader, desk centrifuge, adjustable pipette, micro glass cuvette/96 well flat-bottom plate, cell ultrasonic crusher, ice, distilled water.

Procedure:

I. Sample preparation:

1. Collect bacteria or cells into the centrifuge tube, discard supernatant after centrifugation. According to the proportion of bacteria or cells number (10^4): Extract solution volume (mL) of 500-1000-1 to extract. It is suggested that 5 million of bacteria or cell amount with 0.5mL of Extract solution. Split the bacteria or cell with ultrasonication (placed on ice, ultrasonic power 200W, working time 3s, interval 7s, repeat for 30 times). Centrifuge at 8000 g for 10 minutes at 4℃ to remove insoluble materials, and take the supernatant on ice before testing.
2. Ultrasonication working time could be prolonged properly if samples are fungi, bacteria or other microorganisms with cell walls.

II. Determination procedure:

1. Preheat the Spectrophotometer/Microplate reader for 30 minutes, adjust wavelength to 510 nm, set spectrophotometer counter to zero with distilled water.

2. Add reagents with the following list:

Tel: 86-010-50973105 https://www.solarbio.net E-mail: info@solarbio.com

Reagent (μL)	Test tube (A_T)	Blank tube (A_B)	Standard tube (A_S)
Sample	20	-	-
Distilled water	-	20	-
Standard solution (0.5 μmol/mL)	-	-	20
Reagent I	40	40	40
Reagent II	120	120	120
Reagent III	20	20	20

Mix thoroughly, place at 25℃ for 10 minutes. Take 200μL of reaction solution in micro glass cuvette/96 well flat-bottom plate. Measure absorbance at 510 nm, recorded as A_T, A_B, and A_S. $\Delta A_T = A_T - A_B$, $\Delta A_S = A_S - A_B$. Blank tube and standard tube only need to test once or twice.

III. Cell Iron Content Calculations

1) Bacteria/cells number

Cell iron content (ng/10^4 cell) $=(Cs \times \Delta A_T \div \Delta A_S) \times V_E \times 10^3 \times 55.845 \div 500 = 27.922 \times \Delta A_T \div \Delta A_S$

2) Protein concentration

Cell iron content (ng/mg prot) $=(Cs \times \Delta A_T \div \Delta A_S) \times V_E \times 10^3 \times 55.845 \div (Cpr \times V_E) = 27922.5 \times \Delta A_T \div \Delta A_S \div Cpr$

Cs: Concentration of Fe^{3+} standard solution, 0.5μmol/mL;

V_E: Extract solution volume, 0.5 mL;

10^3: Unit conversion factor, 1 μmol=10^3 nmol;

55.845: Relative molecular mass of Fe, 55.845ng/nmol;

500: Total number of bacteria or cells, 5 million;

Cpr: Supernatant sample protein concentration, mg/mL.

Note:

1. If $\Delta A_T < 0.01$, it is recommended to increase sample supernatant size before determination. If $A_T > 0.8$, it is recommended to dilute sample supernatant with distilled water before determination. And modify the calculation formula.
2. Sample supernatant protein concentration needs to be measured by yourself if cell iron content is calculated by protein content.

Experimental example:

1. Take 5 million cells, add 0.5 mL of Extract solution and split with ultrasonication. Centrifuge and take the supernatant. Then operate according to the determination steps, calculate $\Delta A_T = A_T - A_B = 0.100 - 0.048 = 0.052$, $\Delta A_S = A_S - A_B = 0.408 - 0.048 = 0.360$.The result is calculated according to cells numbers:

   Cell iron content (ng/10^4 cell) $=27.922 \times \Delta A_T \div \Delta A_S = 4.033$ng/$10^4$ cell

Related Products:

BC1730/BC1735 Serum Ferri Ion Content Assay Kit

BC2860/BC2865 Serum Total Iron Binding Capacity(TIBC) Assay Kit

BC4350/BC4355 Tissue Iron Content Assay Kit

Tel: 86-010-50973105 https://www.solarbio.net E-mail: info@solarbio.com