Hemoglobin (Hb) 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: BC5585

Size:100T/96S

Components:

Working solution: Liquid 25mL×1. Store at 2-8℃.

Standard: Powder×1. Store at 2-8℃. Add 1mL distilled water to form 10mg/mL standard solution. It could be stored at 2-8℃ for four weeks. Before use, mix 50μL 10mg/mL standard solution and 750μL distilled water to prepare a standard solution of 0.625 mg/mL.

Product Description:

Hemoglobin (Hb) is a cofactor-containing protein with heme as prosthetic group, which plays an important role in transporting and storing oxygen. It is comprised of two alpha and two beta chains. Each chain has a cyclic tetrapyrrole heme containing an iron atom. Oxygen binds to iron atoms and is transported in the blood.

The improved aqueous alkaline method is used to measure Hb concentration. Hb could react with working solution to form colored compound, which has an absorption peak at 400 nm. Changes of the absorbance at 400nm could be measured to reflect Hb content.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/Microplate reader, desk centrifuge, balance, transferpettor, micro glass cuvette/96 well plate, ice and distilled water.

Procedure:

I. Sample preparation

1. **Whole blood/hemolytic blood/plasma/serum:** detect directly. Centrifuge before detecting if there are precipitation in the plasma/serum.

II. Determination

1. Preheat spectrophotometer/microplate reader for 30 min, adjust the wavelength to 400 nm and set spectrophotometer counter to zero with distilled water.
2. Add reagents in 1.5mL EP tube as the following:

Reagent (μL)	Blank tube	Test tube	Standard tube
Distilled water	50	-	-
Sample	-	50	-
Standard	-	-	50
Working solution	200	200	200

Mix thoroughly and stand at room temperature for 5min. Add 200μL mixture into micro glass cuvette/96 well flat-bottom plate and detect the absorbance value at 400 nm, recording

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as A_B, A_T, and A_S. $\Delta A_T=A_T-A_B$. $\Delta A_S=A_S-A_B$. Blank tube and standard tube need to test once or twice.

III. Hb content calculation:

Hb content (mg/mL) = $\Delta A_T \div (\Delta A_S \div C_S) \times F = 0.625 \times \Delta A_T \div \Delta A_S \times F$

C_S: Standard concentration, 0.625 mg/mL;

F: Dilution factor.

Note:

1. If A_T is more than 1.5, it is recommended to dilute the sample with distilled water before determination. And modify the calculation formula.
2. If A_T is less than 0.01 or close to A_B, it is recommended to increase added sample volume before determination. And modify the added volume of blank tube and standard tube at the same time.

Experimental example:

1. Take 50 μL human serum and operate according to the determination steps, calculate ΔA_T= 0.221 -0.056=0.165, ΔA_S=0.298-0.056=0.242. The result is calculated:
   Hb content (mg/mL) = $0.625 \times \Delta A_T \div \Delta A_S \times F$ =0.426 mg/mL.
2. Take 50 μL mouse whole blood and dilute it 80 times with distilled water. Then operate according to the determination steps, calculate ΔA_T= 0.985-0.056=0.929, ΔA_S=0.298-0.056=0.242. The result is calculated:
   Hb content (mg/mL) = $0.625 \times \Delta A_T \div \Delta A_S \times F$ =191.942 mg/mL.

Related Products:

BC1730/BC1735	Serum Ferri Ion Content Assay Kit
BC5410/BC5415	Ferrous Ion Content Assay Kit
BC5590/BC5595	Free Hemoglobin (FHb) Content Assay Kit
BC5600/BC5605	Methemoglobin (MetHb) Content Assay Kit
BC5610/BC5615	Glycated Hemoglobin (GHb) Content Assay Kit

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