Free Hemoglobin (FHb) 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: BC5595

Size: 100T/96S

Components:

Reagent I: Liquid 16mL×1. Store at 2-8℃.

Reagent II: Liquid 16mL×1. Store at 2-8℃.

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

Working solution: Reagent I, Reagent II and Reagent III are mixed by the ratio of 500μL: 500μL: 25μL (1.025mL, 4T) to make working solution according to sample number.

Standard: Powder×1, 5mg hemoglobin. Store at 2-8℃. Add 1mL distilled water to form 5mg/mL standard solution. It could be stored at 2-8℃ for four weeks.

Product Description:

Red blood cells rupture and free hemoglobin (FHb) content increases in the plasma when hemolysis occurs in blood vessels. Plasma FHb concentration increases mildly to moderately in patients with autoimmune hemolytic anemia, sickle cell disease or thalassemia. The patients have hematuria symptoms who suffer from paroxysmal nocturnal hemoglobinuria, paroxysmal cold hemoglobinuria or unstable hemoglobin disease. It is important to detect FHb content for diagnosis of hemolytic diseases.

Heme in hemoglobin has the catalytic function of peroxidase analogues. It could catalyze the oxidation of 4-aminoantipyrine and phenyl amines by H_2O_2 to form purple quinones. It has a characteristic absorption peak at 546 nm, and its color depth is directly proportional to FHb content.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/Microplate reader, constant temperature foster box/water-bath, desk centrifuge, balance, transferpettor, micro glass cuvette/96 well plate, ice and distilled water.

Procedure:

I. Sample preparation

1. **Plasma or other liquids:** detect directly. Centrifuge before detecting if there are precipitation.

II. Determination

1. Preheat spectrophotometer/microplate reader for 30 min, adjust the wavelength to 546 nm and set spectrophotometer counter to zero with distilled water.
2. Standard working solution: Dilute 5mg/mL(5000mg/L) standard solution with distilled water to 156.25, 78.125, 39.063, 19.531, 9.766, 4.883 mg/L for standby.
3. Preheat Working solution for 15 minutes at 37℃.
4. Add reagents in micro glass cuvette/96 well plate as the following:

Reagent (μL)	Blank tube	Test tube	Standard tube
Distilled water	15	-	-
Sample	-	15	-
Standard	-	-	15
Working solution	250	250	250
Mix thoroughly and stand at 37℃ for 5min. Detect the absorbance value at 546 nm, recording 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 curve need to test once or twice.			

III. FHb content calculation:

1. Standard curve

Taking the concentration of each standard solution as the x-axis and its corresponding ΔA_S as the y-axis, draw a standard curve to get the standard equation y = kx + b, and bring ΔA_T into the equation to get x (mg/L).

2. Calculation

FHb content (mg/L) = x×F

F: Dilution factor.

Note:

1. If sample is plasma, please avoid hemolyzing during plasma sampling and separation.
2. If A_T is more than 1, it is recommended to dilute the sample with distilled water before determination. And modify the calculation formula.
3. 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 15 μL rabbit serum and operate according to the determination steps, calculate ΔA_T= 0.195 -0.096=0.099. Bring the result into the standard curve y=0.0056x-0.03 and calculate x=23.036. The result is calculated:
   FHb content (mg/L) = x×F =23.036 mg/L.
2. Take 15 μL horse serum and operate according to the determination steps, calculate ΔA_T= 0.136-0.096=0.040. Bring the result into the standard curve y=0.0056x-0.03 and calculate x=12.5. The result is calculated:
   FHb content (mg/L) = x×F =12.5 mg/L.

References:

[1] Reeder B, Svistunenko D, Cooper C, et al. The radical and redox chemistry of myoglobin and hemoglobin: from in vitro studies to human pathology [J]. Antioxid Redox Signal, 2004, 6(6): 954-966.

Related Products:

BC1730/BC1735	Serum Ferri Ion Content Assay Kit
BC5410/BC5415	Ferrous Ion Content Assay Kit
BC5580/BC5585	Hemoglobin (Hb) Content Assay Kit
BC5600/BC5605	Methemoglobin (MetHb) Content Assay Kit
BC5610/BC5615	Glycated Hemoglobin (GHb) Content Assay Kit

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