Methemoglobin (MetHb) Content Assay Kit

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

Operation Equipment: Spectrophotometer

Cat No: BC5600

Size:50T/48S

Components:

Reagent I: Liquid 50mL×1. Store at 2-8℃.

Reagent II: Liquid 60mL×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:

Methemoglobin (MetHb) is the oxidation of hemoglobin (Hb), which ferrous ions are oxidized to ferric ions in hemoglobin. Hemoglobin can transport oxygen, but methemoglobin cannot. MetHb content can reflect oxygen carrying capacity of blood and red blood cell substitutes. It is important to detect MetHb content for diagnosis of methemoglobinemia and development of red blood cell substitutes.

Hemoglobin binds to oxygen to form oxyhemoglobin and the latter is deoxidized to form deoxyhemoglobin. Rates of each component mass concentration can be calculated by their extinction coefficients and absorptions at 560nm, 576nm and 630nm. After measuring total Hb content, MetHb content of sample can be calculated by the rates of each component.

Reagents and Equipment Required but Not Provided:

Spectrophotometer, desk centrifuge, balance, transferpettor, 1mL glass cuvette, 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

A. Total Hb content

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

Reagent (μL)	Blank tube (A_B)	Test tube (A_{To})	Standard tube (A_S)
Distilled water	200	-	-

Sample	-	200	-
Standard	-	-	200
Reagent I	800	800	800
Mix thoroughly and stand at room temperature for 5min. Add mixture into 1mL glass cuvette and detect the absorbance value at 400 nm, recording as A_B, A_{To}, and A_S. $\Delta A_{To}=A_{To}-A_B$. $\Delta A_S=A_S-A_B$. Blank tube and standard tube need to test once or twice.			

B. MetHb content

1. Preheat spectrophotometer for 30 min, adjust the wavelength to 560nm, 576nm, 630nm and set counter to zero with distilled water.
2. Add reagents in 1.5mL EP tube as the following.

Reagent (μL)	Test tube (A_T)
Sample	20
Reagent II	1000
Mix thoroughly and stand at room temperature for 5min. Add mixture into 1mL glass cuvette and detect the absorbance value at 560nm, 576nm, 630nm, recording as A_{560}, A_{576}, and A_{630}.	

III. MetHb content calculation:

1. Total Hb content calculation:

Total Hb content (mg/mL) = $\Delta A_{To} \div (\Delta A_S \div C_S) \times F = 0.625 \times \Delta A_{To} \div \Delta A_S \times F$

C_S: Standard concentration, 0.625 mg/mL;

F: Dilution factor.

2. MetHb content calculation:

$[DeoxyHb]=(1.3687\times A_{560}-0.7451\times A_{576}-0.7091\times A_{630}) \times 10^{-4} \div 4$

$[OxyHb]=(-0.7292\times A_{560}+1.0098\times A_{576}-0.3722\times A_{630}) \times 10^{-4} \div 4$

$[MetHb]=(-0.3854\times A_{560}+0.1856\times A_{576}+2.8609\times A_{630}) \times 10^{-4} \div 4$

MetHb (%) =[MetHb]÷([MetHb]+[OxyHb]+[DeoxyHb]) ×100%

MetHb content(mg/mL) = Total Hb content × MetHb (%)

DeoxyHb: Deoxyhemoglobin; OxyHb: Oxyhemoglobin; MetHb (%): the rate of MetHb content in the samples.

Note:

1. If A_{To} is more than 1.0, it is recommended to dilute the sample with distilled water before determination. And modify the calculation formula.
2. If A_{To} 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 rabbit erythrocytes to detect the absorbance value at 560nm, 576nm, 630nm and dilute it

100 times with distilled water to detect total Hb content. And calculate ΔA_{To}=0.537-0.007=0.530,

ΔA_S=0.369-0.007=0.362; A_{560}=0.741, A_{576}=1.218, A_{630}=0.104. The result is calculated:

(1) Total Hb contant (mg/mL) = $0.625\times\Delta A_{To}\div\Delta A_S\times F$ =91.51 mg/mL;

(2) MetHb (%) =0.2380÷ (0.2380+0.6509+0.0329)×100%=25.82%;

(3) MetHb content(mg/mL) = Total Hb content × MetHb (%) =23.627 mg/mL.

2. Take rabbit whole blood to detect the absorbance value at 560nm, 576nm, 630nm and dilute it 160 times with distilled water to detect total Hb content. And calculate ΔA_{To}=0.570-0.007=0.563, ΔA_S=0.369-0.007=0.362; A_{560}=1.111, A_{576}=1.650, A_{630}=0.072. The result is calculated:

(1) Total Hb contant (mg/mL) = $0.625\times\Delta A_{To}\div\Delta A_S\times F$ =155.525 mg/mL;

(2) MetHb (%) =0.084÷ (0.2402+0.8292+0.0840) ×100%=7.283%;

(3) MetHb content(mg/mL) = Total Hb content × MetHb (%) =11.33 mg/mL.

3. Take mouse whole blood to detect the absorbance value at 560nm, 576nm, 630nm and dilute it 160 times with distilled water to detect total Hb content. And calculate ΔA_{To}=0.787-0.007=0.780, ΔA_S=0.369-0.007=0.362; A_{560}=1.518, A_{576}=1.821, A_{630}=0.373. The result is calculated:

(1) Total Hb contant (mg/mL) = $0.625\times\Delta A_{To}\div\Delta A_S\times F$ =215.47 mg/mL;

(2) MetHb (%) =0.8201÷ (0.4562+0.5931+0.8201) ×100%=43.86%;

(3) MetHb content(mg/mL) = Total Hb content × MetHb (%) =95.51 mg/mL.

Related Products:

BC1730/BC1735	Serum Ferri Ion Content Assay Kit
BC5410/BC5415	Ferrous Ion Content Assay Kit
BC5580/BC5585	Hemoglobin (Hb) Content Assay Kit
BC5590/BC5595	Free Hemoglobin (FHb) Content Assay Kit
BC5610/BC5615	Glycated Hemoglobin (GHb) Content Assay Kit

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