SunLong Biotech Co.,LTD
Tel: 0086-571-56623320 Fax:0086-571-56623318
E-mail:sales@sunlongbiotech.com
www.sunlongbiotech.com

# Methemoglobin (MetHb) 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:** AK0756-100T-96S

**Size:** 100T/96S

**Components:** Please carefully check the volume of the reagent and the volume in the bottle before use. If you have any questions, please contact Sunlong staff in time.

| Reagent name | Size | Preservation condition |
|---|---|---|
| Reagent I | Liquid 25 mL×1 | 2-8℃ storage |
| Reagent II | Liquid 30 mL×1 | 2-8℃ storage |
| Standard | Powder×1 | 2-8℃ storage |

**Standard:** 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/Microplate reader, desk centrifuge, transferpettor, micro glass cuvette/96 well plate, ice and distilled water.

**Procedure:**

**I. Sample preparation**

**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/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 ($A_B$) | Test tube ($A_{To}$) | Standard tube ($A_S$) |
|---|---|---|---|
| Distilled water | 50 | - | - |
| Sample | - | 50 | - |
| Standard | - | - | 50 |
| Reagent I | 200 | 200 | 200 |
| Mix thoroughly and stand at room temperature for 5min. Add mixture into micro glass cuvette /96 well plate 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/microplate reader for 30 min, adjust the wavelength to 560nm, 576nm, 630nm and set spectrophotometer counter to zero with distilled water.
2. Add reagents in 1.5mL EP tube as the following.

| Reagent (μL) | Test tube ($A_T$) |
|---|---|
| Sample | 5 |
| Reagent II | 250 |
| Mix thoroughly and stand at room temperature for 5min. Add mixture into micro glass cuvette/96 well plate 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; 100: Percentage unit conversion factor.

**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 50 times with distilled water to detect total Hb content. And calculate $\Delta A_{To}$=0.899-0.053=0.846, $\Delta A_S$=0.326-0.053=0.273; $A_{560}$=0.573, $A_{576}$=0.999, $A_{630}$=0.069. The result is calculated:
   (1) Total Hb contant (mg/mL) = $0.625\times\Delta A_{To}\div\Delta A_S\times F$ =96.84 mg/mL;
   (2) MetHb (%) =0.1620÷ (-0.0090+0.5653+0.1620)×100%=22.55%;
   (3) MetHb content(mg/mL) = Total Hb content × MetHb (%) =21.837 mg/mL.
2. Take rabbit erythrocytes to detect the absorbance value at 560nm, 576nm, 630nm and dilute it 80 times with distilled water to detect total Hb content. And calculate $\Delta A_{To}$=0.906-0.053=0.853, $\Delta A_S$=0.326-0.053=0.273; $A_{560}$=0.998, $A_{576}$=1.760, $A_{630}$=0.076. The result is calculated:
   (1) Total Hb contant (mg/mL) = $0.625\times\Delta A_{To}\div\Delta A_S\times F$ =156.23 mg/mL;
   (2) MetHb (%) =0.1595÷ (0.0007+1.0212+0.1595) ×100%=13.5%;
   (3) MetHb content(mg/mL) = Total Hb content × MetHb (%) =21.09 mg/mL.

**References：**

[1] Li J, Li Z, Zhu Y, Peng H, Du Z, Ru S, Wang W. Bisphenol S remodels red blood cell membrane lipids by alteringplasma lipid levels, causing the risk of venous thrombosis in SD rats and zebrafish embryos. Environ Int. 2023Dec;182:108331. doi: 10.1016/j.envint.2023.108331. Epub 2023 Nov 21. PMID: 37995390.