Methaemoglobin Assay Kit

(Cat/No.:BC142 Size:100T/96S,50T/48S)

1. Composition and preparation: (Shelf life of the kit: 6 months)

Reagent composition	Component	50T/48 samples	100T/96 samples	Storage
Reagent 1	Hemoglobin assay **concentrated solution**	1 mL × 2 vials	1 mL × 4 vials	4℃
	Before use, dilute the concentrated solution with double-distilled water at a ratio of 1:99 (100-fold dilution) to prepare the **hemoglobin assay working solution**. Prepare freshly before use. The prepared reagent can be stored at 2–8 °C protected from light for 1 month.			
Reagent 2	Methemoglobin assay **concentrated solution**	30 mL × 1 bottle	60 mL × 1 bottle	4℃
	Before use, dilute the concentrated solution with double-distilled water at a ratio of 1:9 (10-fold dilution) to prepare the **methemoglobin assay working solution**. The prepared solution can be stored for 6 months.			
Reagent 3	Reduced hemoglobin calibration powder	1 vial	1 vial	4 °C protected from light
	Reduced hemoglobin calibration diluent	1 mL × 1 vial	1 mL × 1 vial	4℃
	Before use, dissolve one vial of powder with one vial of diluent to prepare **Reagent 3 working solution**. Prepare freshly before use and protect from light.			
Reagent 4	Methemoglobin calibration powder	1 vial	1 vial	4 °C protected from light
	Methemoglobin calibration diluent	1 mL × 1 vial	1 mL × 1 vial	4℃
	Before use, dissolve one vial of powder with one vial of diluent to prepare **Reagent 4 working solution**. Prepare freshly before use and protect from light.			

2. Detection significance

After nitric oxide diffuses into the blood in vivo, it rapidly binds to oxyhemoglobin to form methemoglobin (MetHb). Changes in NO are correlated with changes in MetHb;

therefore, the detection of whole-blood MetHb has received widespread clinical attention in recent years. Meanwhile, determination of methemoglobin levels in blood is of great significance for the diagnosis and treatment of toxic diseases caused by exposure to aromatic amines and nitro compounds.

3. Assay principle

Methemoglobin has a characteristic absorption peak at 630 nm, while methemoglobin (MetHb) and reduced hemoglobin (Hb) have identical optical density values at 602 nm. Based on this relationship, the methemoglobin content can be calculated using the corresponding formulas.

4. Required instruments and reagents

Visible spectrophotometer with 1 cm path-length cuvettes, distilled water, test tubes or centrifuge tubes, vortex mixer.

5. Operation procedure

1. **Preparation of anticoagulated whole blood:** Immediately add whole blood into a heparin anticoagulant tube, cap tightly, and gently invert to mix.
2. **Determination of hemoglobin content:** Take 0.01 mL whole blood and add to 2.5 mL of the 100-fold diluted Reagent 1 working solution (hemoglobin assay working solution), mix well, stand for 5 minutes, zero with double-distilled water using a 1 cm path length, and measure absorbance at 540 nm.
3. **Determination of methemoglobin:** Take 0.05 mL whole blood, add 2.5 mL of the diluted Reagent 2 working solution (methemoglobin assay working solution), mix well, stand for 5 minutes, zero with double-distilled water using a 1 cm path length, and measure absorbance at 630 nm and 602 nm. If a dual-wavelength spectrophotometer is not available, first measure absorbance at 630 nm and then at 602 nm. Be sure to carefully note the tube numbering to avoid errors.

6. Calculation formulas

①. Calculation of hemoglobin content:

$$\underset{(g/L)}{\text{Hemoglobin content}} = A_{540nm} \times 367.7$$

②. Calculation of methemoglobin percentage:

$$MetHb\% = \frac{A_{630nm} - r \times A_{602nm}}{A_{602nm} \times (R - r)} \times 100\%$$

③. Calculation of methemoglobin content:

$$\underset{(g/L)}{\text{Methemoglobin content}} = MetHb\% \times \underset{(g/L)}{\text{Hemoglobin content}}$$

[Note 1] A_{630} is the absorbance value of the sample at 630 nm; A_{602} is the absorbance value of the sample at 602 nm.

[Note 2] R and r are constants, **R = 1.81; r = 0.14.** (Constants determined by our laboratory)

Thus, the calculation formulas can be simplified as:

①. Methemoglobin percentage:

$$\text{MetHb\%} = \frac{A_{630nm} - 0.14 \times A_{602nm}}{A_{602nm} \times 1.67} \times 100\%$$

②. Calculation of methemoglobin content:

$$\text{Methemoglobin content (g/L)} = \text{MetHb\%} \times \text{Hemoglobin content (g/L)}$$

7. Calculation examples

①. Take whole blood from a smoker, immediately add it into a heparin anticoagulant tube, cap tightly, and gently invert to mix.

②. Take 0.01 mL of the whole blood and add to 2.5 mL of the 100-fold diluted Reagent 1 working solution (hemoglobin assay working solution), mix well, stand for 5 minutes, measure absorbance at 540 nm using a 1 cm path length with double-distilled water as blank. The measured absorbance value is 0.373. The hemoglobin content can be calculated using the formula:

Hemoglobin content calculation:

$$\text{Hemoglobin content (g/L)} = 0.373 \times 367.7 = 137.15 \text{g/L}$$

③. Take another 0.05 mL of the whole blood, add 2.5 mL of the diluted Reagent 2 working solution (methemoglobin assay working solution), mix well, stand for 5 minutes, zero with double-distilled water using a 1 cm path length, measure absorbance values of 0.226 at 602 nm and 0.066 at 630 nm. The **methemoglobin percentage** can be calculated using the formula:

Methemoglobin percentage calculation:

$$\text{MetHb\%} = \frac{A_{630nm} - r \times A_{602nm}}{A_{602nm} \times (R-r)} \times 100\%$$

$$= \frac{0.066 - 0.14 \times 0.226}{0.226 \times 1.67} \times 100\% = 9.10\%$$

Methemoglobin content calculation:

$$\text{Methemoglobin content (g/L)} = \text{MetHb\%} \times \text{Hemoglobin content (g/L)}$$

$$= 0.091 \times 137.15$$

$$= 12.486 \text{g/L}$$

8. Precautions

①. The experiment should be completed within 1 hour after blood collection; otherwise, the results may be affected, as reductase enzymes in red blood cells can gradually reduce methemoglobin, leading to lower results. Samples can be stored frozen below −20 °C. If a large number of samples are collected, whole blood may be aliquoted into several small tubes. One tube can be used for each test, while the remaining tubes are stored below −20 °C for repeat testing without affecting results.

②. This assay can be performed using a dual-wavelength spectrophotometer to simultaneously measure absorbance at 602 nm and 630 nm. It can also be performed using a single-wavelength spectrophotometer by first measuring absorbance at 630 nm and then at 602 nm. Be careful to avoid mislabeling tubes.

③. If the spectrophotometer does not have dual-wavelength scanning capability, the standard curve may be omitted and the constants determined by our laboratory can be used directly: **R = 1.81; r = 0.14**.

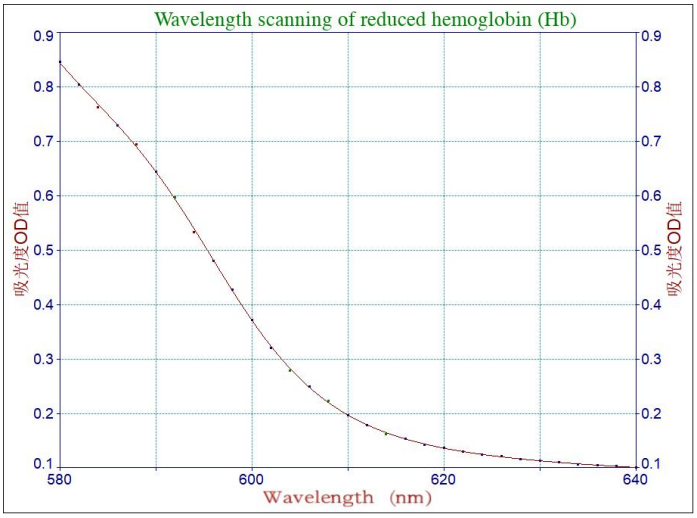
④. All glassware and equipment used must be clean.

9. Scanning of characteristic absorption peaks

○ **Reduced hemoglobin wavelength scanning curve:**

Added component	Reduced hemoglobin tube
Whole blood (mL)	0.02
Reagent 2 working solution (mL)	2.50
Reagent 3 working solution (mL)	0.05

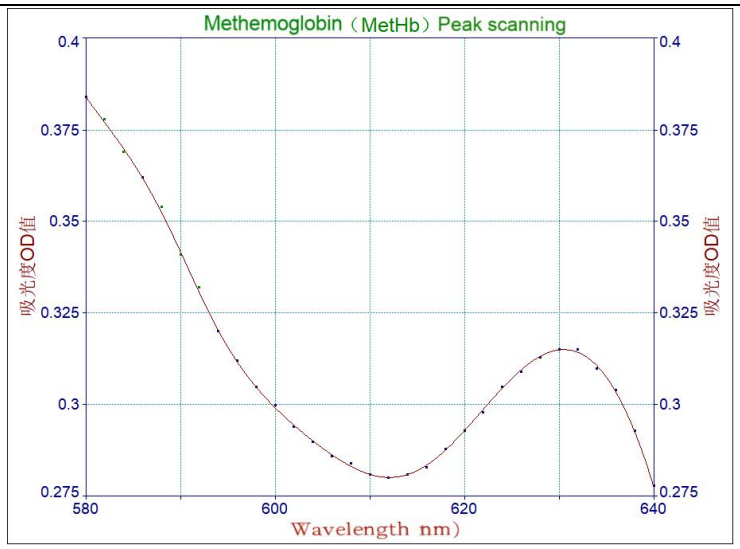
Mix well, stand for 5 minutes, zero with double-distilled water using a 1 cm path length, and perform wavelength scanning.



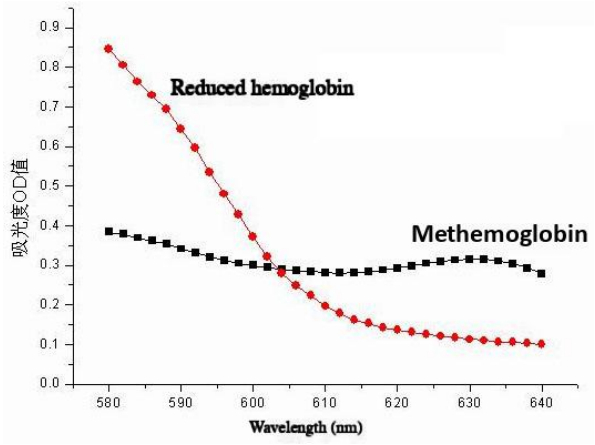
○ **Methemoglobin wavelength scanning procedure:**

Added component	Methemoglobin tube
Whole blood (mL)	0.02
Reagent 2 working solution (mL)	2.50
Reagent 4 working solution (mL)	0.05

Mix well, stand for 5 minutes, zero with double-distilled water using a 1 cm path length, and perform wavelength scanning.



○ **Curve overlap:**



The wavelength scanning curves of methemoglobin (MetHb) and reduced hemoglobin (Hb) intersect at 602 nm, indicating that methemoglobin (MetHb) and reduced hemoglobin (Hb) have equal optical density values at 602 nm.