(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS!)

Methemoglobin (MetHb) Content Assay Kit

Catalog No.: BC00164

Size: 100T

Please read the instructions carefully before use. If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

✉ Email (Sale) order@enkilife.com

✉ Email (Techsupport) techsupport@enkilife.com

☎ Tel: 0086-27-87002838

Website: www.enkilife.com

Shelf life: Please refer to the label on the outer package.

Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Basic Information

Product Name	Methemoglobin (MetHb) Content Assay Kit
Detection Method	Colorimetric
Sample Type	Whole blood
Assay Type	Quantitative
Detection Instrument	Spectrophotometer (optimal detection wavelength 602 nm)

Product Introduction

After nitric oxide diffuses into the bloodstream, it quickly binds with oxyhemoglobin to form methemoglobin (MetHb). Changes in NO levels are correlated with MetHb levels; therefore, whole blood MetHb testing has received widespread clinical attention in recent years. Furthermore, the determination of methemoglobin levels in the blood is of significant importance for the diagnosis and treatment of poisoning diseases caused by exposure to aromatic amino and nitro compounds.

Principle

Methemoglobin has a characteristic absorption peak at a wavelength of 630 nm, while the optical density values of methemoglobin (MetHb) and dehydrohemoglobin (Hb) are equal at a wavelength of 602 nm. This relationship can be used to calculate the methemoglobin content using the corresponding formula.

1. Reduced hemoglobin wavelength scanning curve

Reagents	Reduced hemoglobin tube
Whole blood (mL)	0.02
Reagent 2 application solution (mL)	2.50
Reagent 3 Application Solution (mL)	0.05
Mix well, let stand for 5 minutes, use a 1cm optical path, zero with double-distilled water, and perform wavelength scanning.	

2. Methemoglobin wavelength scanning curve

Reagents	Reduced hemoglobin tube
Whole blood (mL)	0.02
Reagent 2 application solution (mL)	2.50
Reagent 4 Application Solution (mL)	0.05
Mix well, let stand for 5 minutes, use a 1cm optical path, zero with double-distilled water, and perform wavelength scanning.	

3. Curve splicing

The wavelength scanning curves of methemoglobin (MetHb) and dehydroglobin (Hb) intersect at 602 nm, meaning that the optical density values of methemoglobin (MetHb) and dehydroglobin (Hb) are equal at a wavelength of 602 nm.

Components

Component	Name	Size	Storage
Reagent 1	Hemoglobin assay concentrate	1 mL × 4 vials	2~8℃
	Dilute the concentrated solution with double-distilled water at a ratio of 1:99 (100 times dilution) to prepare the hemoglobin assay solution. Prepare the solution immediately before use. Store the prepared reagent at 2–8°C protected from light; it can be stored for one month.		
Reagent 2	Methemoglobin assay concentrate	60 mL × 1 bottle	2~8℃
	When using, dilute the concentrate to double-distilled water at a ratio of 1:9, i.e., a 10-fold dilution, to prepare the application solution for methemoglobin assay. The prepared solution can be stored for 6 months.		
Reagent 3	Reduced hemoglobin curve powder	1 vial	2~8℃, away from light
	Reduced hemoglobin curve powder dilution	1 mL × 1 vial	2~8℃
	Before use, add one vial of diluent to each vial of powder to prepare reagent solution 3. Prepare the solution immediately before use and keep away from light.		
Reagent 4	Methemoglobin curve powder	1 vial	2~8℃, away from light

	Methemoglobin curve powder dilution	1 mL × 1 vial	2~8℃
	Before use, add one vial of diluent to each vial of powder to prepare reagent solution 4. Prepare the solution immediately before use and keep away from light.		

Storage

The kit has a shelf life of 6 months.

Preparation before the experiment

Sample processing

Preparation of anticoagulated whole blood: Take whole blood and immediately add it to a heparin anticoagulation tube, cap and seal, and gently invert to mix.

Operation process

1. Determination of hemoglobin content: Take 0.01 mL of whole blood and 2.5 mL of 100-fold diluted reagent solution (hemoglobin determination solution), mix well, let stand for 5 minutes, and measure the absorbance value of each tube at 540 nm with a 1 cm optical path and double distilled water as the zero point.
2. Methemoglobin assay: Take 0.05 mL of whole blood, add 2.5 mL of reagent dilution working solution (i.e., methemoglobin assay working solution), mix well, let stand for 5 minutes, then zero the instrument with a 1 cm optical path and double-distilled water, and measure the absorbance values at 630 nm and 602 nm. If a dual-wavelength spectrophotometer is not available, you can first measure the absorbance value at 630 nm for each tube, and then measure the absorbance value at 602 nm for each tube. However, be sure to ensure that the tube numbers are correct.

Result calculation

1. Calculation of Hemoglobin Content:

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

2. Calculation of Methemoglobin Percentage:

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

3. Calculation of methemoglobin content:

$$\underset{(g/L)}{\text{MetHb Content}} = \text{MetHb\%} \times \underset{(g/L)}{\text{Hb Content}}$$

Annotation

A_{630nm} refers to the absorbance value of the sample at a wavelength of 630 nm;

A_{602nm} refers to the absorbance value of the sample at a wavelength of 602nm;

Both R and r are constants, R = 1.81; r = 0.14.

Precautions

1. The experiment should be completed within 1 hour after blood collection, otherwise it will affect the test results, because the reductase in red blood cells can gradually reduce methemoglobin, thus lowering the results. However, it can be frozen and stored below -20°C. If there is a large amount of blood, the whole blood can be divided into several small tubes. One small tube can be used for each test, and the remaining tubes can still be stored below -20°C for repeated testing. The results will not be affected.
2. This experiment can be performed using a dual-wavelength spectrophotometer, which can simultaneously measure absorbance values at 602 nm and 630 nm for calculation. Alternatively, a single-wavelength spectrophotometer can be used; in this case, the absorbance value at 630 nm for each tube can be measured first, followed by the absorbance value at 602 nm. However, it is crucial to ensure that the tube numbers are correct.

3. The utensils used must be clean.
4. This product is for research purposes only and must not be used for clinical diagnosis. Do not take it.