Methemoglobin (MetHb) Assay Kit

Catalog No.: abx092001

Size: 100 tests

Storage: Store at 2-8 °C.

Application: For qualitative detection of Methemoglobin **(**MetHb) in animal whole blood samples.

Introduction

Methemoglobin (MetHb) is hemoglobin in the form of a metalloprotein, in which the iron in the heme group exists in the oxidised Fe^{3+} (ferric) state, rather than the reduced Fe^{2+} (ferrous) state of normal hemoglobin. Consequently, MetHb is unable to bind and transport oxygen to tissues. In human blood, trace amounts of MetHb are produced spontaneously. However, in the event MetHb becomes present in excessive quantities, the blood assumes an abnormally dark blue or brown colour. Under basal physiological conditions, The NADH-dependent enzyme methemoglobin reductase is responsible for converting MetHb back to hemoglobin.

Kit Components

1. Reagent A: 4 × 1 ml
2. Reagent B: 60 ml
3. Reagent C: 1 vial
4. Reagent C diluent buffer: 1 ml
5. Reagent D: 1 vial
6. Reagent D diluent buffer: 1 ml

Material Required But Not Provided

1. Microplate reader or spectrophotometer (wavelength: 540, 602 and 630 nm)
2. Pipette and disposable pipette tips
3. Test tubes
4. Vortex mixer
5. Distilled water

Protocol

Reagent Preparation

- **Reagent A working solution:** Dilute Reagent A 100-fold with double-distilled to prepare the Reagent A working solution. Unused Reagent A working solution can be stored at 2-8 °C in the dark for up to 1 month.
- **Reagent B working solution:** Dilute Reagent B 10-fold with double-distilled water to prepare the Reagent B working solution. Unused Reagent B working solution can be stored at 2-8 °C in the dark for up to 6 months.
- **Reagent C working solution:** Reconstitute the vial of Reagent C with 1 ml of Reagent C diluent buffer. Take care to avoid exposure to light.
- **Reagent D working solution:** Reconstitute the vial of Reagent D with 1 ml of Reagent D diluent buffer. Take care to avoid exposure to light.

Sample Preparation

- **Whole blood:** Bring to room temperature prior to use. Add whole blood to a heparin anticoagulant tube immediately, seal, and then mix via gentle inversion.

Assay Procedure

Determination of Hemoglobin (Hb) content

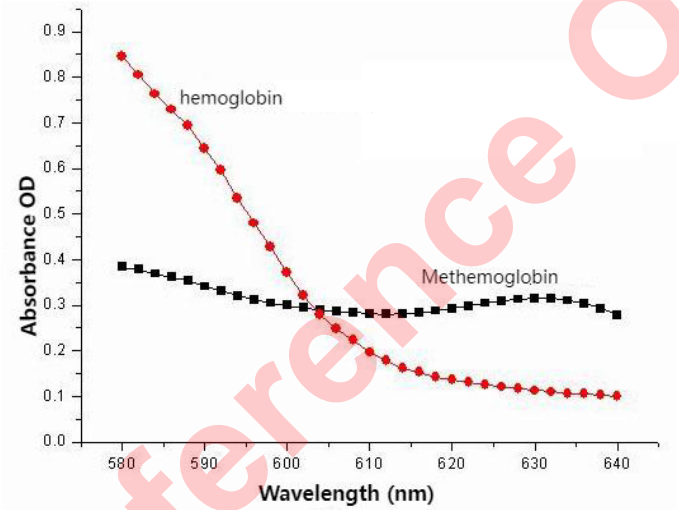
1. Add 0.01 ml of whole blood and 2.5 ml of Reagent A working solution to each test sample vial.
2. Vortex each vial to mix thoroughly and allow to stand for 5 minutes.
3. Use a vial of distilled water to zero the spectrophotometer.
4. Read the O.D. absorbance of each vial at 540 nm, 1 cm optical path in a spectrophotometer.

Determination of Methemoglobin (MetHb) content

1. Add 0.05 ml of whole blood and 2.5 ml of Reagent B working solution to each test sample vial.
2. Vortex each vial to mix thoroughly and allow to stand for 5 minutes.
3. Use a vial of distilled water to zero the spectrophotometer.
4. Read the O.D. absorbance of each vial at 602 nm and 630 nm, 1 cm optical path in a spectrophotometer.
5. If there is no dual-wavelength function on the spectrophotometer, measure the absorbance value initially at 630 nm, followed by the absorbance value at 602 nm.

Wavelength scanning – standard curve

1. To a reducing hemoglobin standard vial, add 0.02 ml of whole blood, 2.5 ml of Reagent B working solution, and 0.05 ml of Reagent C working solution.
2. To a methemoglobin standard vial, add 0.02 ml of whole blood, 2.5 ml of Reagent B working solution, and 0.05 ml of Reagent D working solution.
3. Vortex each vial to mix thoroughly and allow to stand for 5 minutes.
4. Use a vial of distilled water to zero the spectrophotometer, using a 1 cm optical path.
5. Perform wavelength scanning between 580 nm and 640 nm.
6. The reducing hemoglobin absorption curve and methemoglobin absorption curve should intersect at around 602 nm, as shown in the graph below.



Calculations

1. Hemoglobin (Hb) concentration in grams Hb per L:

$$\mathbf{Hb\ (g/L)} = \mathbf{A_{540}} \times \mathbf{367.7}$$

2. Methemoglobin (MetHb) percentage (%):

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

3. Methemoglobin (MetHb) concentration in grams MetHb per L:

$$\mathbf{MetHb\ (g/L)} = \mathbf{MetHb\ (\%)} \times \mathbf{Hb\ (g/L)}$$

where

$\mathbf{A_{540}}$	the absorbance value at wavelength 540 nm
$\mathbf{A_{602}}$	the absorbance value at wavelength 602 nm
$\mathbf{A_{630}}$	the absorbance value at wavelength 630 nm
$\mathbf{R}$	R constant value (1.81)
$\mathbf{r}$	r constant value (0.14)