

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

Endogenous CO Content Assay Kit

Catalog No.: BC00163

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	Endogenous CO Content Assay Kit
Detection Method	Colorimetric
Sample Type	Serum, plasma
Assay Type	Quantitative
Detection Instrument	Spectrophotometer (optimal detection wavelength 540 nm, 568nm, 581nm)

Product Introduction

Carbon monoxide (CO) is a small-molecule gaseous substance very similar to nitric oxide (NO). Extensive research since the 1990s has revealed that endogenous CO in organisms not only participates in information transmission in the central nervous system as a novel neurotransmitter, but also possesses various biological functions such as vasodilation, inhibition of vascular smooth muscle and endothelial proliferation, inhibition of platelet aggregation, regulation of hormone secretion, and inhibition of apoptosis, playing an important role in various physiological and pathological states of the body.

Principle

Based on the difference in absorption spectra between carboxyhemoglobin (COHb) and deoxyhemoglobin (Hb), two wavelengths with the largest difference in COHb absorbance and zero difference in Hb absorbance were selected. The absorbance difference (ΔOD) of the sample at these two wavelengths was measured using dual-wavelength spectrophotometry. The percentage concentration of COHb was then calculated using a standard curve, and the endogenous carbon monoxide (CO) content was calculated by substituting the results into the formula.

Components

Component	Name	Size	Storage
Reagent 1	Hemolytic agents	100 mL × 1 bottle	2~8°C
Reagent 2	Buffer solution	100 mL × 3 bottles	2~8°C
Reagent 3	Carboxyhemoglobin assay reagent	Powder × 10 tubes	2~8°C, away from light
	Before use, add 23 mL of reagent buffer II to each bottle of test powder, cap and invert to mix until completely dissolved to prepare the working solution for carboxyhemoglobin assay. Prepare only the amount needed for each use, and use within 2 hours. Store in a black bag and protect from light.		
Reagent 4	Reducing agent	Powder × 6 tubes	2~8°C, away from light
Reagent 5	Hemoglobin (Hb) assay concentrate	2 mL × 1 bottle	2~8°C, away from light
	Dilute the concentrated solution with 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 more than one month.		

Storage

The kit has a shelf life of 6 months.

Preparation before the experiment

Sample processing

1. Preparation of hemolyzed blood: Take 0.01 mL of anticoagulated whole blood, add 1.2 mL of hemolysing agent (reagent 1), mix well, and let stand for 5 minutes to allow complete hemolysis.
2. Preparation of test sample: Take 0.1 mL of the prepared hemolyzed blood, add 0.1 mL of the plasma to be tested, mix and prepare the test sample.

3. Preparation of control sample: Take 0.1 mL of the prepared hemolyzed blood, add 0.1 mL of the double-distilled water to be tested, mix and prepare the control sample.

Carboxyhemoglobin (COHb) percentage concentration curve

1. Preparation of saturated carboxyhemoglobin and saturated reduced hemoglobin
 - (1) Take 10 μ L of heparin-anticoagulated whole blood from a healthy non-smoker, add 1.2 mL of reagent-1 hemolysate, mix well, and let stand for 5 minutes to allow complete hemolysis.
 - (2) Take two portions, 0.2 mL each, and add them to two stoppered test tubes. Then add 2.3 mL of reagent buffer solution to each tube and mix well.
 - (3) In a fume hood, pure carbon monoxide (CO) is passed through one of the test tubes for 15 minutes, followed by nitrogen gas for 20 minutes, to obtain a saturated COHb solution.
 - (4) Pass oxygen (O₂) into another test tube continuously for 20 minutes to obtain a saturated HbO₂ solution.
2. You can prepare your own COHb percentage concentration curve, or refer to the COHb percentage concentration curve provided in the instruction manual.

Pipe number	1	2	3	4	5	6
Saturated COHb solution (mL)	0	0.25	0.50	1.00	2.00	2.50
Saturated HbO ₂ solution (mL)	2.50	2.25	2.00	1.50	0.50	0
Reagent 4 Reducing Powder	1	1	1	1	1	1
Mix well, let stand for 10 minutes, zero the instrument with reagent two, and measure the absorbance at 568 nm and 581 nm with a 1 cm optical path length.						
COHb percentage concentration	0%	10%	20%	40%	80%	100%
Δ OD (A ₅₆₈ - A ₅₈₁)	0	0.122	0.251	0.481	0.984	1.213

Note: According to the fitted curve, the regression equation is $y = 0.822x + 0.001$; where A₅₆₈ is the wavelength with the largest difference in COHb absorbance; A₅₈₁ is the wavelength with zero difference in Hb absorbance; the absolute absorbance Δ OD value = Δ OD of test tube (A₅₆₈ - A₅₈₁) - Δ OD of control tube (A₅₆₈ - A₅₈₁); x is the absolute absorbance value of Δ OD (A₅₆₈ - A₅₈₁), and y

is the percentage concentration of carboxyhemoglobin .

Operation process

1. Determination of hemoglobin Hb in serum (plasma): Take 0.05 mL of sample (serum, plasma) and 2.5 mL of hemoglobin assay solution (i.e., 100 times dilution of the concentrated solution of reagent five), mix well, let stand for 5 minutes, zero with 1 cm optical path distilled water, and measure the absorbance value of each tube at 540 nm.
2. Determination of carboxyhemoglobin in serum (plasma)

Reagents (mL)	Test tube	Control tube
Pre-processed plasma test sample	0.2	--
Pre-treated plasma control sample	--	0.2
Carboxyhemoglobin assay working solution	2.3	2.3
Mix well, let stand for 10 minutes, zero the reagent buffer solution, measure the absorbance at 568nm and 581nm for each tube with a 1cm optical path, and calculate the absolute absorbance value for each sample.		

Result calculation

1. Calculation of carboxyhemoglobin percentage (COHb%) (refer to the standard curve provided in this manual).

$$\text{COHb\%} = [0.822 \times + 0.001] \times 100\%$$

2. Calculation of hemoglobin (Hb)

$$\text{Hemoglobin g/L (Hb(g/L))} = \text{Absorbance at 540 nm} \times 73.54$$

3. Calculation of carbon monoxide (CO) content in serum (plasma)

$$\text{CO Content } (\mu\text{mol/L}) = \frac{\text{COHb\%} \times \text{Hb(g/L)} \times 10^6 \times 4}{64456} \times \text{Protein Concentration of the Test Sample (gprot/L)}$$

Annotation

64456 is the molecular weight of Hb;

4 indicates that one Hb combines with four CO.

Precautions

This product is for research use only and must not be used for clinical diagnosis. Do not take it.