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

Endogenous CO Content Assay Kit

Catalog No.: BC00162

Size: 50T

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	Tissue
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 (Δ OD) 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℃
Reagent 2	Buffer solution	100 mL × 2 bottles	2~8℃
Reagent 3	Carboxyhemoglobin assay reagent	Powder × 6 tubes	2~8℃, away from light
	Before use, add 23 mL of reagent buffer 2 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℃, away from light
Reagent 5	Hemoglobin (Hb) assay concentrate	2 mL × 1 bottle	2~8℃, 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.		
Reagent 6	Coomassie Brilliant Blue Concentrate	30 mL × 1 bottle	2~8℃
	Dilute with water at a ratio of 1:4 before use.		
Reagent 7	Protein standard solution (0.524 g/L)	0.1 mL × 1 bottle	-20℃

Storage

The kit has a shelf life of 6 months.

Preparation before the experiment

Sample processing

Accurately weigh the tissue and add physiological saline or reagent-hemolysing agent at a ratio of

weight (g): volume (mL) = 1:9. If you are only measuring COHb, you can prepare a 10% tissue homogenate using reagent-hemolysing agent. If you are measuring other indicators at the same time, you can prepare a 10% tissue homogenate using physiological saline. Centrifuge at 2500 rpm for 10 minutes, collect the supernatant, and prepare for testing.

Carboxyhemoglobin (COHb) percentage concentration curve

1. Preparation of saturated carboxyhemoglobin and saturated reduced hemoglobin
   (1) Take 10 μL 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 (O_2) into another test tube continuously for 20 minutes to obtain a saturated HbO_2 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%
△ 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; A_{581} is the wavelength with zero

difference in Hb absorbance; x is the absolute absorbance value of △OD (A_{568} - A_{581}), and y is the percentage concentration of carboxyhemoglobin.

Operation process

1. Hemoglobin (Hb) determination: Take 0.01 mL of sample (whole blood) and 2.5 mL of 100-fold diluted reagent 5 application solution (hemoglobin determination application solution), 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. Carboxyhemoglobin (COHb) determination: Take 0.2 mL of pretreated sample (whole blood lysed after pretreatment), add 2.3 mL of reagent Hb assay working solution, and mix well. After standing for 10 minutes, zero the buffer solution and measure the absorbance at 568 nm and 581 nm with a 1 cm optical path.
3. Determination of tissue protein

Reagents (mL)	Blank tube	Standard tube	Test tube
Distilled water	0.05	--	--
Standard solution	--	0.05	--
Tissue homogenate sample	--	--	0.05
Coomassie Brilliant Blue Application Solution	3	3	3
Mix well, let stand at room temperature for 10 minutes, wavelength 595nm, optical path 1cm, zero with distilled water, and measure the OD value of each tube.			

Result calculation

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

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

2. Calculation of hemoglobin (Hb)

$$\text{Hemoglobin g/L (Hb(g/L))} = \text{Absorbance at 540 nm} \times 367.7$$

3. Calculation of carbon monoxide (CO) content in tissue

$$\text{Tissue Homogenate Protein Concentration（g/L）} = \frac{A_{test} - A_{blank}}{A_{standard} - A_{blank}} \times \underset{（0.524\ g/L）}{\text{Standard Solution Concentration}} \times \text{Dilution Factor}$$

4. Calculation of Carbon Monoxide (CO) Content in Tissue

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

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.