

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

Cytochrome b5 Content Assay Kit

Catalog No.: BC00182

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
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🌐 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	Cytochrome b5 Content Assay Kit
Detection Method	Colorimetric
Assay Type	Quantitative
Sample Type	Tissue
Detection Instrument	Spectrophotometer (424 nm, 490 nm)

Product Introduction

Cytochrome P450 enzymes are a group of isoenzymes mainly distributed in the liver and play a vital role in the metabolism of exogenous substances, especially drugs and toxicants. Cytochrome P450 and cytochrome b5 are two heme proteins of the P450 enzyme system, and changes in their ratio are closely correlated with the metabolic activity of P450.

Principle

After oxidized cytochrome b5 is reduced by sodium dithionite, it exhibits maximum absorption at 424 nm. The content of cytochrome b5 can be calculated by measuring the absorbance difference between 424 nm and 490 nm.

Components

No.	Components	Size	Notes
Reagent 1	Powder	1 vial	Store at 4°C. Add 100 mL of distilled water before use and dissolve completely.
Reagent 2	Liquid	1 bottle	Store at 4 °C
Reagent 3	Powder	1 vial	Store at 4 °C

Working Solution Preparation: Wear disposable gloves, carefully open the cap of Reagent 3, add 50 mL of Reagent 2, and dissolve thoroughly. Store at 4°C away from light for up to one week.

Storage

Store as instructed, valid for 3 months.

Preparation

Visible spectrophotometer, general centrifuge, high-speed centrifuge, adjustable pipette, 1 mL glass cuvette, distilled water.

Operation process

1. Cytochrome b5 Extraction:

Removal of macromolecular substances such as mitochondria and cell nuclei: Weigh 0.5 g of tissue, add 1 mL of Reagent 1, and fully grind and homogenize in an ice water bath.

Centrifuge at 10,000 g and 4 °C for 30 minutes, then transfer the supernatant into a high-speed centrifuge tube.

Crude microsome preparation: Centrifuge at 100,000 g and 4 °C for 60 minutes, and discard the supernatant.

Removal of impurities including hemoglobin: Add 1 mL of Reagent 1 to the precipitate obtained in the previous step, seal the tube tightly, and shake vigorously for full dissolution. Centrifuge at 100,000 g and 4 °C for 30 minutes, then discard the supernatant.

Test solution preparation: Add 0.5 mL of Reagent 2 to the precipitate retained above, tighten the cap and shake thoroughly to dissolve. The prepared solution shall be determined on the same day.

2. Operation Table

	Assay tube	Blank tube
Distilled water (μL)		50
Test solution (μL)	50	
Working solution (μL)	1000	1000

Allow to stand at room temperature for 2 minutes, then read the absorbance at 424 nm as A_1 and at 490 nm as A_2 .

Note: Only one blank tube is required.

Calculation

- a. Calculated based on the protein concentration of the sample.

$$\text{Cytochrome b5 Content (nmol/mg)} = \frac{(\Delta A_{\text{Assay}} - \Delta A_{\text{Blank}}) \times V_{\text{Total}}}{\epsilon \times d \times C_{\text{pr}} \times V_{\text{Sample}}}$$

$$= 0.123 \times (\Delta A_{\text{Assay}} - \Delta A_{\text{Blank}}) \div C_{\text{pr}}$$

- b. Calculated based on sample fresh weight.

$$\text{Cytochrome b5 Content (nmol/g)} = \frac{(\Delta A_{\text{Assay}} - \Delta A_{\text{Blank}}) \times V_{\text{Total}} \times V_{\text{Test}}}{\epsilon \times d \times V_{\text{Sample}} \times W}$$

$$= 0.0614 \times (\Delta A_{\text{Assay}} - \Delta A_{\text{Blank}}) \div W$$

Annotation:

V_{Total} : Total volume of reaction system, 1.05 mL

V_{Sample} : Volume of sample added, 0.05 mL

V_{Test} : Total volume of test solution, 0.5 mL

C_{pr} : Sample protein concentration, mg/mL

W : Sample mass, g

d : Cuvette light path length, 1 cm

ϵ : Molar extinction coefficient of reduced cytochrome b5, 171×10^{-3}

Precautions

1. Wear a lab coat and disposable gloves during operation.

2. This product is intended for scientific research use only by professionals and must not be used for clinical diagnosis or treatment, in food or drugs, or stored in ordinary residences.