



Cytochrome b5

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1034

(Version 1.1C)

Detection and Quantification of Cytochrome b5 Content in Tissue extracts, Cell lysate Samples.

For research use only. Not for diagnostic or therapeutic procedures.

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I. INTRODUCTION

Cytochrome b5 is a membrane-bound hemoprotein that enhances the catalytic efficiency of some P450 isoforms. Cytochrome P450 and cytochrome b5 are two hemoglobin proteins, the ratio change are closely related to the P450 metabolic. Oxidized cytochrome b5 was reduced by dithionite and have the maximum absorption peak at 424 nm, we can calculate the amount of cytochrome b5 by measuring the difference of absorbance at 424 nm and 490 nm.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Reagent I	Powder x 2	4 °C
Reagent II	25 ml x 3	4 °C
Reagent III	Powder x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

Note:

Reagent I: add 100 ml distilled water to dissolve before use, store at 4 °C.

Reagent III: add 20 ml Reagent II to dissolve before use, store at 4 °C, keep in dark.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 424 nm and 490 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Ice
7. Centrifuge
8. Timer

IV. SAMPLE PREPARATION

1. For tissue samples

Weigh 0.5 g tissue, homogenize with 1 ml Reagent I on ice, centrifuged at 10,000g 4 °C for 30 minutes, take the supernatant into a new centrifuge tube. Centrifuged at 100,000g 4 °C for 60 minutes, discard the supernatant. Add 1 ml Reagent I to the precipitation, shock. Centrifuged at 100,000g 4 °C for 30 minutes, discard the supernatant. Add 0.5 ml Reagent II to the precipitation, shock. Keep it on ice for detection.

V. ASSAY PROCEDURE

Warm Reagent III to the room temperature.

Add following reagents in the microplate:

Reagent	Sample	Blank
Sample	10 μ l	--
Distilled water	--	10 μ l
Reagent III	200 μ l	200 μ l
Mix, stand at room temperature for 2 minutes, record absorbance measured at 424 nm and 490 nm.		

1) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

1. According to the protein concentration of sample

$$\begin{aligned}\text{Cytochrome b5 (nmol/mg)} &= [(OD_{\text{Sample424}} - OD_{\text{Sample490}}) - (OD_{\text{Blank424}} - OD_{\text{Blank490}})] / (\epsilon \times \\ &\quad d) \times V_{\text{Total}} / (V_{\text{Sample}} \times C_{\text{Protein}}) \\ &= 0.195 \times [(OD_{\text{Sample424}} - OD_{\text{Sample490}}) - (OD_{\text{Blank424}} - \\ &\quad OD_{\text{Blank490}})] / C_{\text{Protein}}\end{aligned}$$

2. According to the weight of sample

$$\begin{aligned}\text{Cytochrome b5 (nmol/g)} &= [(OD_{\text{Sample424}} - OD_{\text{Sample490}}) - (OD_{\text{Blank424}} - OD_{\text{Blank490}})] / (\epsilon \times d) \\ &\quad \times V_{\text{Total}} \times (V_{\text{Assay}} / V_{\text{Sample}}) / W \\ &= 0.0975 \times [(OD_{\text{Sample424}} - OD_{\text{Sample490}}) - (OD_{\text{Blank424}} - OD_{\text{Blank490}})] \\ &\quad / W\end{aligned}$$

ϵ : molar extinction coefficient of reductive Cytochrome b5, $171 \times 10^3 \text{ L/nmol/cm}$;

d : the optical path of 96-Well microplate, 0.63 cm;

C_{Protein} : the protein concentration, mg/ml;

W : the weight of sample, g;

V_{Total} : the total volume of the enzymatic reaction, 0.21 ml = $2.1 \times 10^{-4} \text{ L}$;

V_{Sample} : the volume of sample, 0.01 ml;

V_{Assay} : the volume of Reagent I, 0.5 ml.

VII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

VIII. NOTES