Cytochrome b5Microplate 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.

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 424nm, we can calculate the amount of cytochrome b5 by measuring the difference of absorbance at 424nm and 490nm.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Reagent I	Powderx 2	4 °C
Reagent II	25 mlx 3	4 °C
Reagent III	Powderx 1	4 °C
Plate Adhesive Strips	3 Strips	
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Note:

Reagent I: add 100 mldistilled 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

Weigh0.5g tissue, homogenize with 1 ml Reagent I on ice, centrifuged at 10,000g 4°C for 30minutes, take the supernatant into a new centrifuge tube. Centrifuged at 100,000g 4°C for 60minutes, discard the supernatant. Add 1 ml Reagent Ito the precipitation, shock. Centrifuged at 100,000g 4°C for 30minutes, discard the supernatant. Add 0.5 ml Reagent IIto the precipitation,shock.Keep it on ice for detection.

V. ASSAY PROCEDURE

WarmReagent IIIto the room temperature.

Add following reagents in the microplate:

Reagent	Sample	Blank
Sample	10 µl	--
Distilled water	--	10 µl
Reagent III	200 µl	200 µl
Mix, stand at room temperature for2minutes, 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

Cytochrome b5 (nmol/mg) =[($OD_{Sample424}$ -$OD_{Sample490}$) -($OD_{Blank424}$ - $OD_{Blank490}$)] / (ε× d)×V_{Total} / (V_{Sample}×$C_{Protein}$)

= 0.195× [($OD_{Sample424}$ - $OD_{Sample490}$) - ($OD_{Blank424}$ - $OD_{Blank490}$)]/ $C_{Protein}$

2. According to the weight of sample

Cytochrome b5 (nmol/g) = [($OD_{Sample424}$ - $OD_{Sample490}$) - ($OD_{Blank424}$ - $OD_{Blank490}$)] / (ε× d)×V_{Total}×(V_{Assay} / V_{Sample}) / W

= 0.0975× [($OD_{Sample424}$ - $OD_{Sample490}$) - ($OD_{Blank424}$ - $OD_{Blank490}$)] / W

ε: molar extinction coefficient of reductive Cytochrome b5,171× 10^{-3}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 × 10^{-4} 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 towww.cohesionbio.com or contact us at techsupport@cohesionbio.com

VIII. NOTES