NADPH-Cytochrome c Reductase Microplate Assay Kit

Catalog # AS0030

Detection and Quantification of NADPH-Cytochrome c Reductase Activity in Tissue extracts, Cell lysate Samples.

This instruction must be read in its entirety before using this product.

For research use only, Not for use in diagnostic procedures.

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I. INTRODUCTION

Eukaryotic NADPH-Cytochrome c reductase (NADPH-cytochrome P450 reductase, EC 1.6.2.4) is a flavoprotein localized to the endoplasmic reticulum. It transfers electrons from NADPH to several oxygenases, the most important of which is the cytochrome P450 family of enzymes, responsible for xenobiotic metabolism. NADPH-cytochrome c reductase is widely used as an endoplasmic reticulum marker and as a biomarker of ecological pollution and dietary lipid uptake.

This kit is based on a colorimetric assay that measures the reduction of cytochrome c by NADPH-Cytochrome c reductase in the presence of NADPH. The reduction of cytochrome c results in the formation of distinct bands in the absorption spectrum and the increase in absorbance at 550 nm is measured with time.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer I	30 mlx 4	4 °C
Assay Buffer II	30 mlx 1	4 °C
Reaction Buffer	20 mlx 1	4 °C
Substrate I	Powderx 1	4 °C, keep in dark
Substrate II	Powderx 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

Note:

Substrate I: add 1 mldistilled water to dissolve before use, store at 4 °C.

Substrate II: add 1 mldistilled water to dissolve before use, store at 4 °C.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 550 nm
2. Distilled water
3. Pipettor
4. Pipette tips
5. Mortar
6. Ice
7. Centrifuge
8. Timer

IV. SAMPLE PREPARATION

1.For tissue samples

Weighout 0.5 g tissue, homogenize with 1 ml Assay Buffer I on ice, centrifuged at 10,000g 4°C for 20minutes, take the supernatant into a new centrifuge tube. Centrifuged at 100,000g 4°C for 60minutes, discard the supernatant. Add 1 ml Assay Buffer Ito the precipitation, mix and vortex, centrifuged at 100,000g 4°C for 30minutes, discard the supernatant. Add 0.5 ml Assay BufferIIto the precipitation,mix and vortex.Keep it on ice for detection.

V. ASSAY PROCEDURE

Add following reagents intothe microplate:

Reagent	Sample	Control
Reaction Buffer	170µl	170 µl
Substrate I	10 µl	10 µl
Substrate II	10 µl	10 µl
Distilled water	--	10 µl
Sample	10 µl	
Mix, measured at 550 nm immediately and recordthe absorbance of the first 10th second and 130th second.		

VI. CALCULATION

Unit Definition: One unit of NCR activity is the enzyme that generates 1 nmol of the reduction of cytochrome c per minute.

1. According to the protein concentration of sample

$$NCR\ (U/mg) = [(OD_{Sample(130S)} - OD_{Sample(10S)}) - (OD_{Control\ (130S)} - OD_{Control\ (10S)})] / (\varepsilon \times d) \times V_{Total} / (V_{Sample} \times C_{Protein}) / T$$

$$= 872.6 \times [(OD_{Sample(130S)} - OD_{Sample(10S)}) - (OD_{Control\ (130S)} - OD_{Control\ (10S)})] / C_{Protein}$$

2. According to the weight of sample

$$NCR\ (U/g) = [(OD_{Sample(130S)} - OD_{Sample(10S)}) - (OD_{Control\ (130S)} - OD_{Control\ (10S)})] / (\varepsilon \times d) \times V_{Total} / (V_{Sample} \times W / V_{Assay}) / T$$

$$= 436.3 \times [(OD_{Sample(130S)} - OD_{Sample(10S)}) - (OD_{Control\ (130S)} - OD_{Control\ (10S)})] / W$$

ε: molar extinction coefficient of reductive Cytochrome c,0.0191L/μmol/cm;

d: the optical pathof 96-Well microplate, 0.6 cm;

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

W: the weight of sample, g;

V_{Total}: the total volume of the enzymatic reaction, 0.2 ml;

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

V_{Assay}: the volume of Assay BufferII, 0.5 ml;

T: the reaction time, 2 minutes.

VII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.sabbiotech.cn or contact us at techcn@signalwayantibody.com

VIII. NOTES