NADPH-Cytochrome c Reductase

Microplate Assay Kit

Cat #: orb390736 (manual)

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

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

Biorbyt Ltd
7 Signet Court, Swann Road, Cambridge, CB5 8LA. United Kingdom
Email: info@biorbyt.com | Phone: +44 (0)1223 859-353 | Fax: +44 (0)1223 280-240

Biorbyt LLC
68 TW Alexander Drive, Durham, NC 27709. United States
Email: info@biorbyt.com | Phone: +1 (415) 906-5211 | Fax: +1 (415) 651-8558

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.

KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer I	30 ml x 4	4 °C
Assay Buffer II	30 ml x 2	4 °C
Reaction Buffer	20 ml x 1	4 °C
Substrate I	Powder x 1	4 °C, keep in dark
Substrate II	Powder x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

Note:

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

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

MATERIALS REQUIRED BUT NOT PROVIDED

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

SAMPLE PREPARATION

1. For tissue samples

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

ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent	Sample	Control
Reaction Buffer	170 µl	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 record the absorbance of the first 10th second and 130th second.		

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, 0.0191 L/μmol/cm;

d: the optical path of 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 Buffer II, 0.5 ml;

T: the reaction time, 2 minutes.

Biorbyt Ltd
7 Signet Court, Swann Road, Cambridge, CB5 8LA. United Kingdom
Email: info@biorbyt.com | Phone: +44 (0)1223 859-353 | Fax: +44 (0)1223 280-240

Biorbyt LLC
68 TW Alexander Drive, Durham, NC 27709. United States
Email: info@biorbyt.com | Phone: +1 (415) 906-5211 | Fax: +1 (415) 651-8558