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

NADPH-Cytochrome c Reductase (NCR) Activity Assay Kit

Catalog No.: BC00158

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

☎ Tel: 0086-27-87002838

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	NADPH-Cytochrome c Reductase (NCR) Activity Assay Kit
Detection Method	Colorimetric
Sample Type	Tissues
Assay Type	Quantitative
Detection Instrument	Spectrophotometer (optimal detection wavelength 550 nm)

Product Introduction

Cytochrome P450 enzymes are a group of isoenzymes mainly found in the liver, playing an important role in the metabolism of exogenous substances, especially drugs and toxins. The NCR, as an important member of the P450 enzyme system, catalyzes the reduction and regeneration of oxidized P450.

Principle

NCR catalyzes the reduction of oxidized cytochrome c by NADPH. Reduced cytochrome c has a unique absorption peak at 550 nm. NCR activity is calculated by measuring the rate of increase in absorbance at 550 nm.

Components

Components	Size	Storage
Reagent 1	Liquid × 1 bottle	2~8℃
Reagent 2	Liquid × 1 bottle	2~8℃
Reagent 3	Powder ×1 bottle	-20℃
	Dissolve thoroughly in 2.6 mL of distilled water before use, and store at 2~8℃.	

Reagent 4	Powder ×1 bottle	2~8℃
	Dissolve thoroughly in 550 μL of distilled water before use, and store at 2~8℃.	

Storage

The kit has a shelf life of 6 months.

Preparation before the experiment

Sample processing

Crude enzyme extraction: Weigh approximately 0.5 g of tissue, add 1 mL of pre-cooled reagent one at 4°C, grind thoroughly on ice, centrifuge at 10,000 g, 4°C for 30 min, and transfer the supernatant to an ultracentrifuge tube; centrifuge at 100,000 g, 60 min at 4°C, and discard the supernatant; add 1 mL of reagent one to the precipitate, seal tightly, and shake thoroughly to dissolve, centrifuge at 100,000 g, 30 min, and discard the supernatant; add 0.5 mL of reagent two to the precipitate from the previous step, seal tightly, and shake thoroughly to dissolve, and store at 4°C until analysis.

Operation process

1. Preheat the spectrophotometer for at least 30 minutes, adjust the wavelength to 550 nm, and zero it with distilled water.
2. Reagent 2 should be preheated in a water bath at 37°C for at least 30 minutes.
3. Operating steps:

Reagents(μL)	Blank tube	Test tube
Double-distilled water	50	--
Crude enzyme solution of sample	--	50
Reagent 2	900	900
Reagent 3	50	50

Reagent 4	10	10
Mix quickly, and zero the sample at 550 nm using a 1 mL quartz cuvette and double-distilled water. Measure the absorbance values A_{10} at 10 s and A_{130} at 130 s Calculate: $\Delta A_{test}=A_{10}-A_{130}$.		

Note: When the sample size is large, only one blank tube is needed.

Result calculation

1. Calculated based on sample protein concentration

Unit definition :At 25°C, one unit (1 U) is defined as the amount of protein that produces 1 nmol of reduced cytochrome c per minute.

$$\text{NCR activity (U/mg prot)} = \frac{\Delta A_{test} - \Delta A_{blank}}{\varepsilon \times d} \times V_{reaction\ total} \div (Cpr \times V_{sample}) \div T$$

$$= 529 \times (\Delta A_{test} - \Delta A_{blank}) \div Cpr$$

2. Calculated by sample quality

Unit definition: At 25°C, one unit (1 U) is defined as the amount of enzyme that catalyzes the production of 1 nmol of reduced cytochrome c per minute per gram of sample.

$$\text{NCR activity (U/g)} = \frac{\Delta A_{test} - \Delta A_{blank}}{\varepsilon \times d} \times V_{reaction\ total} \div (W \times V_{sample} \div V_{sample\ total}) \div T$$

$$= 265 \times (\Delta A_{test} - \Delta A_{blank}) \div W$$

Annotation

ε：The molar absorptivity of reduced cytochrome c at 550 nm is 1.91×10^4 L/mol/cm.

d: Cuvette path length (cm), 1 cm;

$V_{reaction\ total}$: Total volume of the reaction system, 1010 μL = 1.01×10^{-3}L

$V_{sample\ total}$: 0.5 mL of the extracted solution added;

V_{sample} : Volume of supernatant added to the reaction system (mL), 50μL = 0.05mL;

10^6 : 1 mol = 1×10^6 μmol

T: Catalytic reaction time, 2 min;

Cpr: Protein concentration in the supernatant (mg/mL), which needs to be determined separately. It is

recommended to use our company's BCA protein content assay kit (BC00006).

W: Sample mass, g;

Precautions

This product is for research purposes only and must not be used for clinical diagnosis. Do not take it.