NADPH-Cytochrome C Reductase (NCR) Activity Assay Kit

Note: It is necessary to predict 2-3 large difference samples before the formal determination.

Operation Equipment: Spectrophotometer/Microplate reader

Cat No: BC2725

Size:100T/96S

Components:

Reagent I: Powder×2. Store at 2-8°C. Add 100mL distilled water to each Reagent I and mix well before use. It can be stored at 2-8°C for four weeks.

Reagent II: Liquid 80mL×1. Store at 2-8°C.

Reagent III: Powder×2. Store at -20°C. Add 520μL distilled water to each Reagent III and mix well before use. It can be stored at -20°C for two weeks after dispensing to avoid repeated freezing and thawing.

Reagent IV: Powder×2. Store at 2-8°C. Add 550μL distilled water to each Reagent IV and mix well before use. It can be stored at -20°C for four weeks after dispensing to avoid repeated freezing and thawing.

Product Description:

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

NCR catalyzes the reduction of oxidized cytochrome C by NADPH to produce reduced cytochrome C, which has a characteristic absorption peak at 550nm. NCR activity is calculated by measuring the rate of increase in absorbance at 550nm.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/Microplate reader, constant temperature foster box/water-bath, desk centrifuge, transferpettor, micro glass cuvette/96-well flat-bottom plate, mortar/homogenizer, ice and distilled water.

Procedure:

I. Sample preparation

1. Remove cell nucleus and mitochondria: weigh about 0.5g of tissue, add 1 mL of pre-cooled reagent I, grind thoroughly on ice, centrifuge at 10000g at 4°C for 30 minutes, take the supernatant, and transfer to an ultracentrifuge tube.
2. Crude microsomes: centrifuge for 60 min at 4°C, 100 000 g, and discard the supernatant.
3. Removal of impurities such as hemoglobin: add 1 mL of reagent I to the precipitate of step 2, and then fully shake to dissolve it after it is tightly capped. Centrifuge at 100 000 g for 30 min, and discard the supernatant.
4. Final microsomes: add 0.5 mL of reagent II to the precipitation of step 3, cover tightly and shake to dissolve it, and store at 4°C for testing.

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II. Determination

1. Preheat spectrophotometer/microplate reader for 30 min, adjust the wavelength to 550 nm and set spectrophotometer counter to zero with distilled water.
2. Keep Reagent II at 37℃ for 30 min according to sample number.
3. Add reagents in 1 mL quartz cuvette as the following:

Reagent (μL)	Test tube (A_T)	Blank tube (A_B)
Distilled Water	-	10
Sample	10	-
Reagent II	180	180
Reagent III	10	10
Reagent IV	10	10

Measure the change in absorbance within 2 minutes at 550nm after mixing quickly. The absorbance value of blank tube at the 10 s and 130 s are recorded as A1 and A2, respectively, ΔA_B = A2-A1. The absorbance value of test tube at the 10 s and 130 s are recorded as A3 and A4, respectively, ΔA_T = A4-A3. Calculate $\Delta A=\Delta A_T-\Delta A_B$. The blank tube only needs to be measured once or twice.

III. Calculation:

A. micro glass cuvette:

1. Protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes production of 1 nmol reduced cytochrome C in the reaction system per minute at 37℃ every mg protein.

NCR activity (U/mg prot) $=\Delta A\div(\varepsilon\times d)\times Vrv\div(Cpr\times Vs)\div T=550\times\Delta A\div Cpr$

2. Sample weight:

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes production of 1 nmol reduced cytochrome C in the reaction system per minute at 25℃ every g sample.

NCR activity (U/g weight) $=\Delta A\div(\varepsilon\times d)\times Vrv\div(W\times Vs\div Ve)\div T=275\times\Delta A\div W$

ε: extinction coefficient, 19100 L/mol/cm=1.91×10^{-5} L/nmol/cm;

d: Light path of cuvette, 1cm;

Vrv: Total reaction volume, 0.00021 L;

Cpr: Crude enzyme protein concentration, mg/mL;

Vs: Crude enzyme volume, 0.01 mL;

T: Reaction time, 2 min;

W: Sample weight, g;

Ve: Extract volume of reagent II, 0.5mL.

B. 96-well flat-bottom plate:

Modify d-1 cm in the above formula to d-0.6 cm (light path of the cuvette) for calculation.

Note:

1. If ΔA_T is close to ΔA_B or ΔA_T is low, it is recommended to increase the sample size before

determination. If $\Delta A_T > 1.5$ or $\Delta A > 1$, it is recommended to dilute the sample with Reagent II before determination. And modify the calculation formula.