

NADP(H) Concentrate Assay Kit

(Cat/No.:BC163 Size:50T/24S)

1. Composition

Reagents	Specifications	Storage
Acidic extract	50mL×1 bottle	4°C
Alkaline extract	50mL×1 bottle	4°C
Reagent 1	15mL×1 bottle	4°C
Reagent 2	Powder×1 vial	-20°C
	Prepare of Reagent 2 solution : When used, add 4mL distilled water, mix well, An endless supply of the reagent is kept at 4 °C for a week	
Reagent 3	Powder×1 vial	-20°C
	Prepare of Reagent 3 solution : When used, add 4mL distilled water, mix well, An endless supply of the reagent is kept at 4 °C for a week	
Reagent 4	Powder×1 bottle	4°C
	Prepare of Reagent 4 solution : When used, add 4mL distilled water, mix well, An endless supply of the reagent is kept at 4 °C for a week	
Reagen 5	1.8mL×1 bottle	4°C
Reagen 6	30mL×1 bottle	4°C
Reagen 7	50mL×1 bottle	4°C

2. Assay Principle

NADP⁺ and NADPH in the sample were extracted with acidic and alkaline extracts, Through the hydrogen transfer of PMS, NADPH reduced MTT to Formazan, and the content of NADPH could be calculated by measuring the absorbance at 570 nm. The content of NADP⁺ was determined by reducing NADP⁺ to NADPH by 6-glucose-phosphate dehydrogenase.

3. Assay significance

Coenzyme II NADPH is widely found in animals, plants, microorganisms and cultured cells, NADP (NADPH + NADP⁺) and NADPH / NADP⁺ ratio can be calculated by NADP⁺ and



NADPH content determination. Changes in this ratio are associated with pentose phosphate

pathway and biosynthesis, as well as antioxidant reactions. NADPH / NADP + ratio is not only

one of the major markers of redox status, but also plays an important role in the regulation of PPP pathway, biosynthesis and antioxidant metabolism.

4. Additional Materials Required

- 1 ·Visible spectrophotometer, Activity (Wavelength: 570nm)
- 2 ·High speed refrigerated centrifuge and tubes, Water bath
- 3 ·Adjustable pipette (5-1000µl) and Tips
- 4 ·1mL glass cuvettes
- 5 ·Absolute ethanol and Distilled water

5. Extraction of NADP⁺ and NADPH

① Extraction of NADP⁺ and NADPH in serum (Blood plasma)

Extraction of NADP⁺ : According to the volume (ml) of serum (Plasma) : The volume of acidic extract (ml) is 1:5-10 (about 0.1 mL of serum (plasma) and 1 mL of acidic extract is added) , and boiled for 5 minutes (cover tightly to prevent water dispersion) , and centrifuged at 4 °C for

10 minutes after cooling in ice bath The supernatant was centrifuged at 4°C for 10 Min, then the supernatant was added into a new centrifuge tube, and then neutralized and mixed with an equal volume of alkaline extract.

Extraction of NADPH: According to the volume (ml) of serum (plasma) : The volume (ml) of alkaline extract (ML) is 1:5-10 (about 0.1 mL of serum (plasma) and 1 mL of alkaline extract is

added) , boil for 5 minutes (cover tightly to prevent water dispersion) , and centrifuge at 4 °C for 10 minutes after cooling in ice bath The supernatant was centrifuged at 4 °C for 10 min. The supernatant was added into a new centrifuge tube, and then neutralized and mixed with the same volume of acidic extract.

② Extraction of NADP⁺ and NADPH in tissue

Extraction of NADP⁺ : According to the Tissue Quality (g) : The volume of the acidic extract (ml) is 1:5-10 (about 0.1 g tissue is taken and 1 mL acidic extract is added) , grind in ice bath and boil for 5 min (cover tightly to prevent water dispersion) , and Centrifugate at 4°C for 10 min after cooling in ice bath The supernatant was centrifuged at 4°C for 10 Min, then the supernatant was

added into a new centrifuge tube, and then neutralized and mixed with an equal volume of alkaline extract.

Extraction of NADPH: According to the Tissue Quality (g) : The volume of alkaline extract



(ml) is 1:5-10 (about 0.1 g tissue is taken and 1 mL alkaline extract is added), the liquid is ground in ice bath and boiled for 5 min (cover tightly to prevent water dispersion), and cooled in ice bath, centrifuged at 4 °C for 10 Min at 10000 G; The supernatant was centrifuged at 4°C for 10 Min.

The supernatant was added into a new centrifuge tube, and then neutralized and mixed with the same volume of acidic extract.

③ Extraction of NADP⁺ and NADPH in cells and bacteria

Extraction of NADP⁺: Cells or bacteria were collected into the centrifuge tube and the supernatant was discarded according to the number of bacteria or cells (10^4): The volume of

acidic extract (ml) was 500-1000:1 (it is suggested that 5 million bacteria or cells should be added with 1 mL of acidic extract), after cooling in the ice bath, the supernatant was centrifuged at 4 °C for 10min, and the supernatant was added into another new centrifuge tube to neutralize the

supernatant by adding an equal volume of alkaline extract, the mixture was centrifuged at 4°C for 10min at 10000g, the supernatant was taken and put on ice to be tested.

Extraction of NADPH: Cells or bacteria were collected into the centrifuge tube and the supernatant was discarded according to the ratio of the number of bacteria or cells (10^4): The volume of alkaline extract (ml) was 500-1000:1 (it is suggested that 5 million bacteria or cells

should be added with 1 ML alkaline extract), after cooling in the ice bath, the supernatant was

centrifuged at 4°C for 10min, and the supernatant was added to another new centrifuge tube, and then neutralized by adding an equal volume of acidic extract, the mixture was centrifuged at 4°C for 10min at 10000g, the supernatant was taken and put on ice to be tested.

6. Operation table

Reagents	Assay	Contrast
Sample (μL)	50	50
Reagent 1 (μL)	250	250
Reagent 2 (μL)	75	75
Reagent 3 (μL)	75	75
Reagent 4 (μL)	75	75
Reagent 5 (μL)	35	35
Reagent 6 (μL)	Mix well, left for 20min at room temperature avoid the light	500
Reagent 6 (μL)	500	
Mix well, left for 5 min, 20000g, centrifuged at room temperature for 5 min, discard the supernatant and take out the precipitate		
Reagent 7 (μL)	1000	1000



Mix well, adjusting zero with distilled water,
measure the absorption value of each tube 570nm with 1 cm diameter cuvette.
Value of contrast is A1 and value of Assay is A2, Calculate $\Delta A = A2 - A1$

Note: ①、Sample and reagent are added in 1.5 ml brown EP tube according to the table below.

The reaction should be kept out of light.

②、Reagent 6 must be added immediately after adding Reagent 5 in Control Tube, and reagent 6 must be added after reacting 20 minutes after adding Reagent 5 in

determination tube.

7. Calculate

(一) Calculate of NADP⁺

1. NADP⁺ in serum (plasma)



$$\text{NADP}^+ \text{ concentration} = \frac{[4.57 \times (\Delta A - 0.062) \times V_1]}{(V_3 \times V_1 \div V_2)} = 91.4 \times (\Delta A - 0.062)$$

(nmol/ml)

2. NADP⁺ in tissue、bacteria and cells

(1) Calculated by sample protein concentration

$$\text{NADP}^+ \text{ concentration} = \frac{[4.57 \times (\Delta A - 0.062) \times V_1]}{(V_1 \times C_{pr})} = 4.57 \times (\Delta A - 0.062) \div C_{pr}$$

(nmol/mgprot)

(2) Calculated by sample fresh weight

$$\text{NADP}^+ \text{ concentration} = \frac{[4.57 \times (\Delta A - 0.062) \times V_1]}{(W \times V_1 \div V_2)} = 9.14 \times (\Delta A - 0.062) \div W$$

(nmol/g tissue)

(3) Calculated by bacteria or cell density

$$\text{NADP}^+ \text{ concentration} = \frac{[4.57 \times (\Delta A - 0.062) \times V_1]}{(500 \times V_1 \div V_2)} = 0.0183 \times (\Delta A - 0.062)$$

(nmol/10⁴ cells)

(二) Calculate of NADPH

1. NADPH in serum (plasma)

$$\text{NADPH concentration} = \frac{[7.2 \times (\Delta A - 0.072) \times V_1]}{(V_3 \times V_1 \div V_2)} = 144 \times (\Delta A - 0.072)$$

(nmol/ml)

2. NADH in tissue、bacteria and cells

(1) Calculated by sample protein concentration

$$\text{NADPH concentration} = \frac{[7.2 \times (\Delta A - 0.072) \times V_1]}{(V_1 \times C_{pr})} = 7.2 \times (\Delta A - 0.072) \div C_{pr}$$

(nmol/mgprot)

(2) Calculated by sample fresh weight

$$\text{NADPH concentration} = \frac{[7.2 \times (\Delta A - 0.072) \times V_1]}{(W \times V_1 \div V_2)} = 14.4 \times (\Delta A - 0.072) \div W$$

(nmol/g tissue)

(3) Calculated by bacteria or cell density

$$\text{NADPH concentration} = \frac{[7.2 \times (\Delta A - 0.072) \times V_1]}{(500 \times V_1 \div V_2)} = 0.0288 \times (\Delta A - 0.072)$$

(nmol/10⁴ cells)



V_1 : Sample volume added to the reaction system, 0.05 mL;

V_2 : The volume of the extract added, 2 mL;

V_3 : The volume of serum (Plasma) added: 0.1 mL;

C_{protein} : Sample protein concentration, mg/mL;

W: Sample quality, g; 500: Total number of cells or bacteria, 5 million

8. Points to attentions

- 1、 If you need to test a large number of samples at one time, you can mix the reagent 1, 2, 3, 4 in proportion, and prepare the mix reagents when you need.
- 2、 Try to avoid light during the reaction.
- 3、 Since each tube needs to be set to a pair, this kit can measure 24 NADP^+ or NADPH in 50 tubes, If both indicators are to be determined, this kit can only determine 12 samples
- 4、 Minimum Detection Limit of the kit is 1 nmol/mL or 1 nmol/g tissue weight or 0.01 nmol/mg prot