NADPase Microplate Assay Kit

User Manual

Catalog # FTA0017

(Version 1.2D)

Detection and Quantification of NADPase activity in Tissue extracts, Cell lysate Samples.

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

I. INTRODUCTION

NADPase mainly present in plant tissue. NADPase is the only enzyme in vivo which catalytic $NADP^+$ degradation to NAD^+, and regulation balance between NAD and NADP together with NADK.

NADPase can catalyze the NAD^+ hydrolysis to $NADP^+$ and reactions of inorganic phosphorus, NADPase activity was measured by measuring the amount of inorganic phosphorus.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer	20 ml x 1	4 °C
Substrate	Powder x 2	-20 °C
Dye Reagent I	Powder x 1	4 °C
Dye Reagent II	Powder x 1	4 °C
Dye Reagent III	20 ml x 1	4 °C
Standard (5 µmol/ml)	1 ml x 1	4 °C
Plate Adhesive Strips	3 Strips	
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Substrate: add 1 ml Reaction Buffer before use.

Dye Reagent: add 1 ml Dye Reagent III into Dye Reagent I and Dye Reagent II respectively for dissolve, then mix 3 Dye Reagents together.

***Note:** It should be yellow. If colorless, the solution is failure. If blue, the solution is polluted. This solution should be prepared before use. It is best to use disposable plastic containers to prepare the solution in order to prevent phosphorus pollution.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 660 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Ice
7. Centrifuge
8. Timer

IV. SAMPLE PREPARATION

1. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay buffer on ice, centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

V. ASSAY PROCEDURE

Add following reagents into the microcentrifuge tubes:

Reagent	Sample	Control	Standard	Blank
Reaction Buffer	140 µl	140 µl	--	--
Substrate	20 µl	20 µl	--	--
Mix, put it in the oven, 37 °C for 5 minutes.				
Sample	40 µl	--	--	--
Distilled water	--	40 µl	--	--
Mix, put it in the oven, 37 °C for 30 minutes. Then put it in boiling water for 5 minutes. When cold, centrifuged at 10000g, room temperature for 5 minutes.				
Add following reagents into the 96-Well microplate:				
Standard	--	--	20 µl	--
Distilled water	--	--	--	20 µl
Supernatant	20 µl	20 µl	--	--
Dye Reagent	180 µl	180 µl	180 µl	180 µl
Mix, room temperature for 30 minutes, record absorbance measured at 660nm.				

Note:

1) It is best to use disposable plastic tube to avoid phosphorus pollution.

2) Perform 2-fold serial dilutions of the top standards to make the standard curve.

3) For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more sample into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

VI. CALCULATION

Unit Definition: One unit of NADPase activity is defined as the enzyme generates 1 μmol of PO_4^{3-} per hour.

1. According to the protein concentration of sample

$$\text{NADPase (U/mg)} = (C_{Standard} \times V_{Total}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times C_{Protein}) / T$$

$$= 50 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

$$\text{NADPase (U/g)} = (C_{Standard} \times V_{Total}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (W \times V_{Sample} / V_{Assay}) / T$$

$$= 50 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / W$$

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

W: the weight of sample, g;

$C_{Standard}$: the concentration of standard, 5 μmol/ml;

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

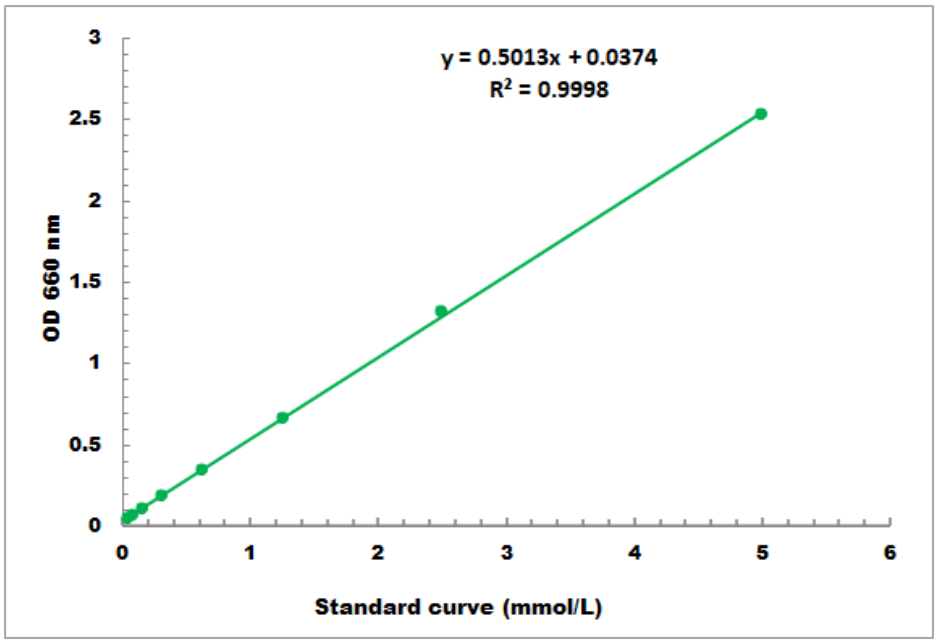
V_{Sample}: the volume of sample, 0.04 ml;

V_{Assay}: the volume of Assay buffer, 1 ml;

T: the reaction time, 0.5 h.

VII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 0.01 µmol/ml - 5 µmol/ml

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES