NADPase Activity

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1017

(Version 1.4D)

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

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

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I. INTRODUCTION

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

NADPase Activity ColorimetricMicroplate Assay Kit provides a simple and direct procedure for measuring NADPase activity in a variety of samples.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 I	30 ml x 4	4 °C
Assay Buffer II	Powder x 1	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 (5mmol/L)	1 ml x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

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. REAGENT PREPARATION

Standard:Perform 2-fold serial dilutions of the top standardsolution using distilled waterto make the standard curve. The concentration of standard curve could be 5/2.5/1.25/0.625/0.312/0.156/0.078mmol/L.

Assay Buffer II: Briefly centrifuge prior to opening. Add 1.2 ml ethanol to dissolve before use. Keep in dark for immediate use or store at -20°C for 3-4 months after reconstitution.

Substrate: Briefly centrifuge prior to opening. Add 1 mlReaction Bufferbefore use.Store at 4 °C for 2-3 weeks or -20°C for 4-5 month after reconstitution.

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 solutionispolluted.This solution should be preparedbefore use.It is best to usedisposable plastic containers to prepare the solution in order to prevent phosphoruspollution.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

V. SAMPLE PREPARATION

1.For tissue samples

Weighout 0.1 g tissue, homogenize with 0.99 mlAssay Buffer Iand 10 µl Assay Buffer II on ice, centrifuged at 8000g 4°C for 10minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

VI. ASSAY PROCEDURE

Add following reagents intothe 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 intothe 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:

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

*Reagents must be added sequentially and should not be premixed prior to addition.

**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 samples into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

Unit Definition: One unit of NADPaseactivityis defined asthe enzyme generates 1μmol of PO_4^{3-}per minute.

1. According to the slope of the standard curve

$$\text{Activity} = \frac{(OD_{Sample}-OD_{Control}) - \text{Intercept}}{\text{Slope}\times T} \times \frac{V_{Standard}}{V_{Sample}} \times n(U/mL)$$

2. According to one point of the standard OD value and concentration

2.1 According to the protein concentration of sample

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

2.3 According to the weight of sample

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

Slope: the absorbance slope of standard curve

n: the dilution factor

$C_{Protein}$: the protein concentration of sample, mg/mL

W: the weight of total sample, g

$C_{Standard}$: the concentration of standard, μmol/mL

$V_{Standard}$: the volume of standard in assay procedure, mL

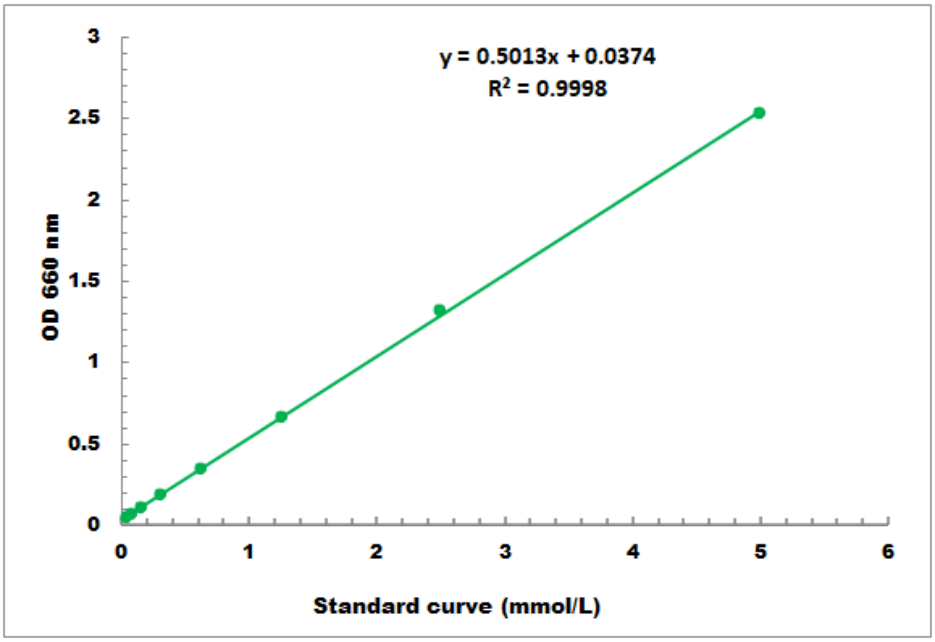
V_{Sample}: the volume of sample in assay procedure, mL

V_{Assay}: the volume of Assay Buffer in sample preparation, mL

T: the reaction time, minute

VIII. TYPICAL DATA

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



Detection Range: 0.01 mmol/L - 5mmol/L