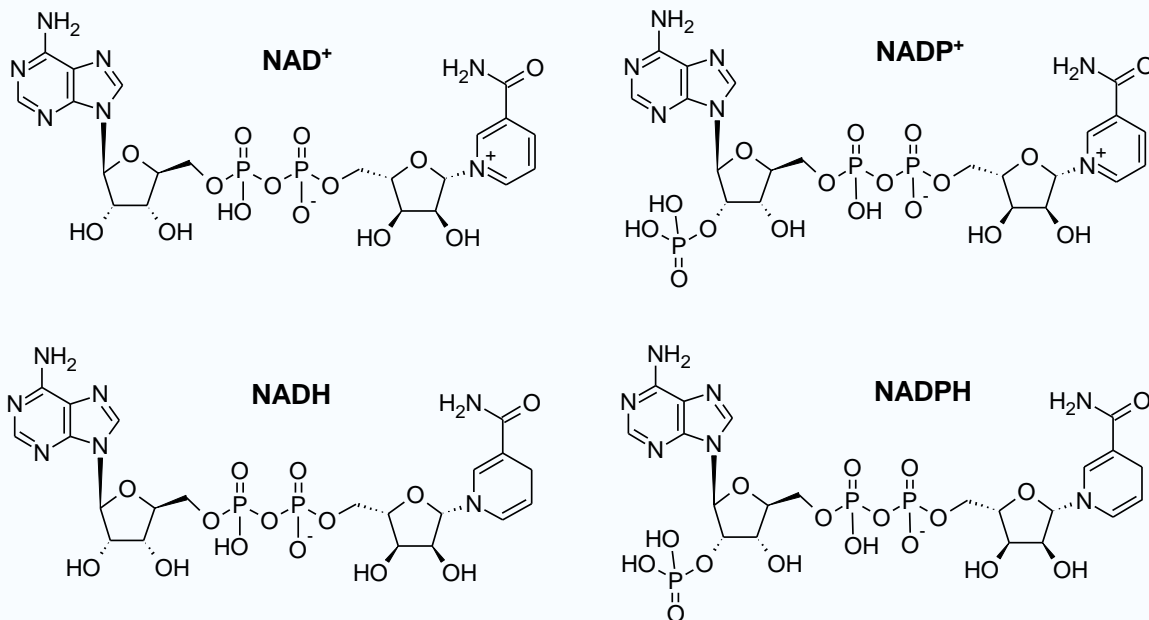


Photometric Detection of Nicotinamide Adenine Dinucleotides

Nicotinamide adenine dinucleotide (NAD⁺) is an important coenzyme found in cells. The coenzyme NAD⁺ was first discovered by Arthur Harden and William Youndin in 1906. It plays key roles as a carrier of electrons and a participant in metabolic redox reactions, as well as in cell signaling. There are two forms of this coenzyme in cells, the non-phosphorylated form NAD⁺ and the phosphorylated form NADP⁺. These two related coenzymes have similar chemistry, but perform different roles in metabolism. When both coenzymes are being discussed, they are referred to collectively as NAD(P)⁺.

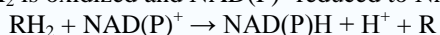


NADH is the reduced form of NAD⁺, and NADPH is the reduced form of NADP⁺. In metabolism, NAD⁺ and NADP⁺ are used as oxidizing agents - they accept electrons from other molecules, whereas NADH and NADPH are reducing agents that donate electrons. In chemical terms, the coenzymes are reactants in hydride transfer reactions. These redox reactions are the primary function of NAD(P)⁺. However, these coenzymes are also used in a range of metabolic processes that do not involve redox reactions, such as second messenger systems and posttranslational modifications.

NAD⁺ can be synthesized from amino acids such as tryptophan. Alternatively, fragments of the coenzyme taken up in the diet or released by reactions that break down the structure of NAD⁺ can be salvaged and then recycled back into the active form. Due to the central role of these coenzymes in metabolism, the enzymes involved in making and using NAD(P)⁺ have been described as promising targets for drug discovery. This may be particularly important in the development of future drugs to exploit the roles of NAD⁺ in the regulation of aging and longevity.

Physical and Chemical Properties

NAD and NADP are stable if stored dry and in the dark. Solutions of NAD(P)⁺ are colorless and stable for about one week at 4 °C with neutral pH, but decompose rapidly in acids. Upon decomposition, they form products that are enzyme inhibitors. Cells use NAD(P)⁺ to accept or donate electrons in redox reactions. During these reactions the reductant RH₂ is oxidized and NAD(P)⁺ reduced to NAD(P)H.



The above reaction involves the removal of two hydrogen atoms in the form of a hydride ion and a proton H⁺ from the reactant R. The proton is released into solution. From the hydride electron pair, one electron is transferred to the positively-charged nitrogen of the nicotinamide ring of NAD(P)⁺, and the other hydrogen transferred to the carbon atom opposite this nitrogen.

Biological Functions

Redox Functions: Nicotinamide adenine dinucleotide is an essential part of cell metabolism. In rat liver, the total amount of NAD⁺ and NADH is approximately 1 μmol^{-1} g wet weight, with about 10-fold less NADP⁺ and NADPH. The actual concentration of NAD⁺ in cell cytoplasm is hard to measure, with recent estimates in red blood cells ranging around 300 μM . However, over 80% of NADH is bound to proteins, so the concentration of free coenzymes is much lower.

The main role of NAD(P)⁺ in metabolism is the transfer of electrons from one redox reaction to another, which is very important in the release of energy from nutrients. The reduced compounds such as glucose are oxidized, releasing energy. The energy is transferred to NAD⁺ by reducing it to NADH, as part of glycolysis and the citric acid cycle. The NADH is then oxidized in turn by the electron transport chain, which pumps protons across a membrane and generates ATP through oxidative phosphorylation. Since the oxidized and reduced forms of nicotinamide adenine dinucleotide are both used in these linked sets of reactions, the cell maintains approximately equal concentrations of NAD⁺ and NADH; the high NAD⁺/NADH ratio allows this coenzyme to act as both an oxidizing and a reducing agent. In contrast, the main role of the phosphorylated form of this coenzyme is as a reducing agent in anabolism, so the NADP⁺/NADPH ratio is kept very low, allowing NADPH to drive redox reactions as a strong reducing agent.

The balance between the oxidized and reduced forms of nicotinamide adenine dinucleotide is called the NAD⁺/NADH ratio. This ratio reflects the redox state of a cell, which controls both the metabolic activity and the health of that cell. Several key enzymes are controlled by the NAD⁺/NADH ratio, including glyceraldehyde 3-phosphate dehydrogenase and pyruvate dehydrogenase. In healthy mammalian tissues, estimates of the NAD⁺/NADH ratio range around 1, so the concentrations of NAD⁺ and NADH are roughly comparable. In contrast, the NADP⁺/NADPH ratio is about 0.005, around 200 times lower than the NAD⁺/NADH ratio, so NADPH is the dominant form of this coenzyme. These different ratios reflect the different metabolic roles of NADH and NADPH.

Non-redox Functions: The coenzyme NAD⁺ can also be consumed in ADP-ribose transfer reactions. For example, some enzymes add the ADP-ribose moiety of this molecule to proteins, in a posttranslational modification called ADP-ribosylation. These reactions are involved in cell signaling and the control of many cell processes, including DNA repair and apoptosis. ADP-ribose can also be transferred to proteins in long branched chains, in a reaction called poly(ADP-ribosyl)ation. This protein modification is carried out by the poly ADP-ribose polymerases. The poly (ADP-ribose) structure is involved in the regulation of several cellular events and is very important in the cell nucleus, in processes such as DNA repair and telomere maintenance.

Other NAD-dependent enzymes include bacterial DNA ligases, which join two DNA ends by using NAD as a substrate to donate an AMP group to the 5' phosphate of one DNA end that is then attacked by the 3' hydroxyl group of the other DNA end, forming a new phosphodiester bond. This is in contrast to eukaryotic DNA ligases, which use ATP to form the DNA-AMP intermediate. NAD⁺ is also consumed by sirtuins, which are NAD-dependent deacetylases, such as Sir2. These enzymes act by transferring an acetyl group from their substrate protein to the ADP-ribose moiety of NAD⁺, which cleaves the coenzyme and releases nicotinamide and O-acetyl-ADP-ribose. The sirtuins seem to be mainly involved in regulating transcription through deacetylating histones and altering nucleosome structure.

NAD⁺ can also be converted into cyclic ADP-ribose by ADP-ribosyl cyclases as part of a second messenger system. The cyclic ADP ribose second messenger molecule acts in calcium signaling by releasing calcium from intracellular stores. It does this by binding to and opening a class of calcium channels called ryanodine receptors.

Binding to Proteins: AD(P)⁺ and NAD(P)H bind to a structural motif named the Rossmann fold. This motif is found in many proteins that bind nucleotides. The structure is composed of three or more parallel beta strands linked by two alpha helices in the topological order beta-alpha-beta-alpha-beta. Because each Rossmann fold binds one nucleotide, binding domains for the dinucleotide NAD⁺ consists of two paired Rossmann folds, with each fold binding one nucleotide moiety of the cofactor. Single Rossmann folds are found in proteins that bind mononucleotides, such as the cofactor FMN.

Despite this similarity in how proteins bind NAD⁺ and NADP⁺, enzymes almost always show a high level of specificity for either one or the other of these coenzymes. This specificity is due to the distinct metabolic roles of the two coenzymes and results from distinct sets of amino acid residues in the coenzyme-binding pocket. For example, in the active site of NADP-dependent enzymes, a specific ionic bond is formed between a basic amino acid side chain and the acidic phosphate group of NADP⁺. Conversely, in NAD-dependent enzymes the charge in this pocket is reversed, preventing NADP⁺ binding. There are a few exceptions to this general rule. For example,

enzymes (such as aldose reductase, glucose-6-phosphate dehydrogenase and methylenetetrahydrofolate reductase) show dual-cofactor specificity in some species.

Detections of NAD/NADH and NADP/NADPH

NAD(P)⁺ absorbs strongly in the ultraviolet due to the adenine base. Peak absorption is at 259 nm, with an extinction coefficient of 16,900 M⁻¹ cm⁻¹. The reduced forms of NAD(P)H also absorb at a higher wavelength, with a second peak in UV absorption at 339 nm that has an extinction coefficient of 6,220 M⁻¹ cm⁻¹. This difference in the ultraviolet absorption spectrums between the oxidized and reduced forms of the coenzymes makes it simple to measure the conversion of one to another in enzyme assays by measuring the amount of UV absorption at 339 nm using a spectrophotometer. However, the UV method suffers from poor selectivity, high background and low sensitivity since many biological substances have strong absorption from 250 to 400 nm. It is quite troublesome to use the UV detection method to detect NAD/NADH and NADP/NADPH when they coexist with other biological molecules that have UV absorption from 250 to 400 nm.

AAT Bioquest has developed a series of NAD/NADH and NADP/NADPH detection kits. Our Amplite™ NAD/NADH and NADP/NADPH detection kits have good selectivity, low background and high sensitivity since we use our proprietary red absorbing or red fluorescent NAD(P) sensors. These new red absorbing or red fluorescent NAD(P) sensors demonstrate strong absorption or strong fluorescence in the range where only few biological molecules have absorption or fluorescence.

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Amplite™ Colorimetric NAD/NADH Assay Kit

Quick Facts at a Glance

Product Number: 15258

Recording Modality:

Instrument Platform:

Kit Size: 400 assays

Absorbance at 576±5 nm

Any absorbance microplate readers with proper filters

Kit Features

Nicotinamide adenine dinucleotide (NAD⁺) and nicotinamide adenine dinucleotide phosphate (NADP⁺) are two important cofactors found in cells. NADH is the reduced form of NAD⁺, and NAD⁺ is the oxidized form of NADH. It forms NADP with the addition of a phosphate group to the 2' position of the adenyl nucleotide through an ester linkage. NADP is used in anabolic biological reactions, such as fatty acid and nucleic acid synthesis, which require NADPH as a reducing agent. In chloroplasts, NADP is an oxidizing agent important in the preliminary reactions of photosynthesis. The NADPH produced by photosynthesis is used as reducing power for the biosynthetic reactions in the Calvin cycle of photosynthesis. The traditional NAD/NADH and NADP/NADPH assays are carried out by monitoring the NADH or NADPH with absorption at 340 nm. This method suffers from low sensitivity and high interference since the assay is run in the UV range that requires expensive quartz microplate. This Amplite™ Colorimetric NAD/NADH Assay Kit provides a convenient method for sensitive detection of NAD, NADH and their ratio. The enzymes in the system specifically recognize NAD/NADH in an enzyme cycling reaction. There is no need to purify NAD/NADH from the sample mix. The enzyme cycling reaction significantly increases detection sensitivity. In addition, this assay has very low background since it is run in the red visible range that significantly reduces the interference from biological samples. The assay has demonstrated high sensitivity and low interference with absorbance at 576±5 nm.

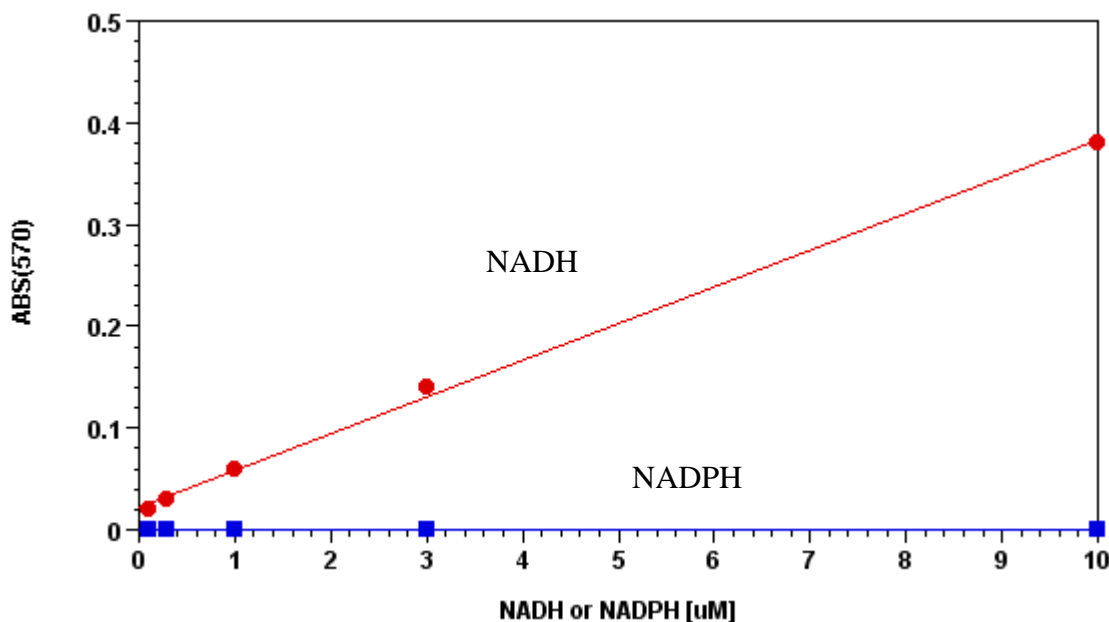


Figure 1. NADH dose response was measured on a 96-well black plate with Amplite™ NAD/NADH Assay Kit using a BMG LabTech NOVOStar microplate reader. As low as 300 nM (30 pmol/well) of NADH was detected with 1hour incubation time (n=3) while there was no response from NADPH.

Amplite™ Colorimetric NADP/NADPH Assay Kit

Quick Facts at a Glance

Product Number: 15260

Kit Size: 400 assays

Recording Modality:

Absorbance at 576±5 nm

Instrument Platform:

Any absorbance microplate readers with proper filters

Kit Features

Nicotinamide adenine dinucleotide (NAD⁺) and nicotinamide adenine dinucleotide phosphate (NADP⁺) are two important cofactors found in cells. NADH is the reduced form of NAD⁺, and NAD⁺ is the oxidized form of NADH. It forms NADP with the addition of a phosphate group to the 2' position of the adenylyl nucleotide through an ester linkage. NADP is used in anabolic biological reactions, such as fatty acid and nucleic acid synthesis, which require NADPH as a reducing agent. In chloroplasts, NADP is an oxidizing agent important in the preliminary reactions of photosynthesis. The NADPH produced by photosynthesis is then used as reducing power for the biosynthetic reactions in the Calvin cycle of photosynthesis. The traditional NAD/NADH and NADP/NADPH assays are carried out by monitoring the NADH or NADPH with absorption at 340 nm. This method suffers from low sensitivity and high interference since the assay is done in the UV range that requires expensive quartz microplate. This Amplite™ Colorimetric NADP/NADPH Assay Kit provides a convenient method for the sensitive detection of NADP, NADPH and their ratio. The enzymes in the system specifically recognize NADP/NADPH in an enzyme cycling reaction. There is no need to purify NADP/NADPH from the sample mix. The enzyme cycling reaction significantly increases detection sensitivity. In addition, this assay has very low background since it is run in the red visible range that significantly reduces the interference from the biological samples. The assay has demonstrated high sensitivity and low interference with absorbance at 576±5 nm.

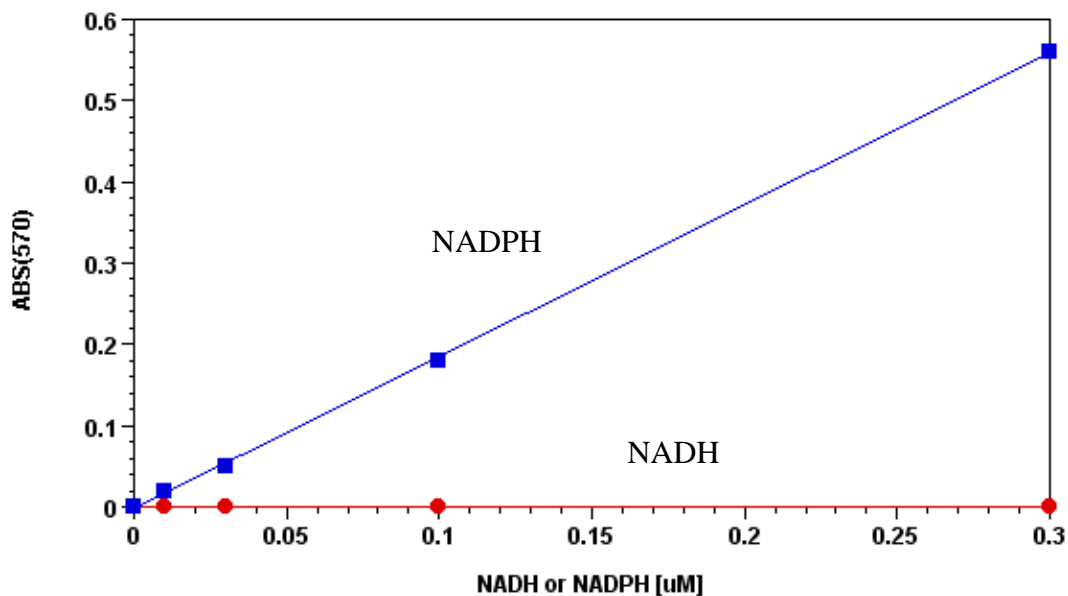


Figure 2 NADPH dose response was measured on a 96-well black plate with Amplite™ NADP/NADPH Assay Kit using a BMG LabTech NOVOStar microplate reader. As low as 100 nM (10 pmol/well) of NADPH was detected with 1hour incubation time (n=3) while there was no response from NADH.

Amplite™ Fluorimetric NADH Assay Kit *Red Fluorescence*

Quick Facts at a Glance

Product Number: 15261

Kit Size: 400 assays

Recording Modality:

Fluorescence setting at Ex/Em = 540/590 nm

Instrument Platform:

Any fluorescence microplate readers with proper filters

Kit Features

Nicotinamide adenine dinucleotide (NAD⁺) and nicotinamide adenine dinucleotide phosphate (NADP⁺) are two important cofactors found in cells. NADH is the reduced form of NAD⁺, the oxidized form of NADH. It forms NADP with the addition of a phosphate group to the 2' position of the adenyl nucleotide through an ester linkage. NADP is used in anabolic biological reactions, such as fatty acid and nucleic acid synthesis, which requires NADPH as a reducing agent. In chloroplasts, NADP is an oxidizing agent important in the preliminary reactions of photosynthesis. The NADPH produced by photosynthesis is used as reducing power for the biosynthetic reactions in the Calvin cycle of photosynthesis. The traditional NAD/NADH and NADP/NADPH assays are done by monitoring the NADH or NADPH with absorption at 340 nm. This method suffers low sensitivity and high interference since the assay is run in the UV range that requires expensive quartz microplate. This Amplite™ Fluorimetric NADH Assay Kit provides a convenient method for the detection of NADH. The enzymes in the system specifically recognize NADH in an enzyme reducing reaction. In addition, this assay has very low background since it is carried out in the red visible range that significantly reduces the interference from the biological samples. The assay has demonstrated high sensitivity and low interference with excitation and emission filters set at 540/590 nm.

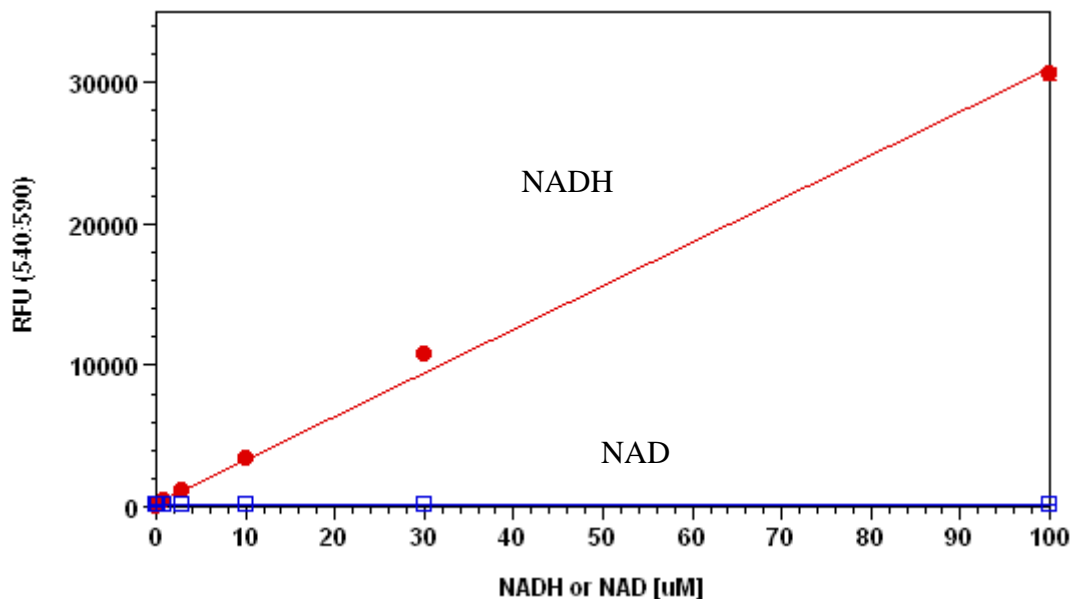


Figure 3 NADH dose response was measured on a 96-well black plate with Amplite™ NADH Assay Kit using a BMG LabTech NOVOSTar microplate reader. As low as 1 µM (10 nmol/well) of NADH was detected with 1 hour incubation time (n=3) while there was no response from NAD.

Amplite™ Fluorimetric NAD/NADH Assay Kit

Red Fluorescence

Quick Facts at a Glance

Product Number: 15257	Kit Size: 400 assays
Recording Modality:	Fluorescence setting at Ex/Em = 540/590 nm
Instrument Platform:	Any fluorescence microplate readers with proper filters

Kit Features

Nicotinamide adenine dinucleotide (NAD⁺) and nicotinamide adenine dinucleotide phosphate (NADP⁺) are two important cofactors found in cells. NADH is the reduced form of NAD⁺, and NAD⁺ is the oxidized form of NADH. It forms NADP with the addition of a phosphate group to the 2' position of the adenyl nucleotide through an ester linkage. NADP is used in anabolic biological reactions, such as fatty acid and nucleic acid synthesis, which require NADPH as a reducing agent. In chloroplasts, NADP is an oxidizing agent important in the preliminary reactions of photosynthesis. The NADPH produced by photosynthesis is then used as reducing power for the biosynthetic reactions in the Calvin cycle of photosynthesis. The traditional NAD/NADH and NADP/NADPH assays are carried out by monitoring the NADH or NADPH with absorption at 340 nm. This method suffers low sensitivity and high interference since the assay is done in the UV range that requires expensive quartz microplate. This Amplite™ Fluorimetric NAD/NADH Assay Kit provides a convenient method for the sensitive detection of NAD, NADH and their ratio. The enzymes in the system specifically recognize NAD/NADH in an enzyme cycling reaction. There is no need to purify NAD/NADH from the sample mix. The enzyme cycling reaction significantly increases the detection sensitivity. In addition, this assay has very low background since it is run in the red visible range that significantly reduces the interference from the biological samples. The assay has demonstrated high sensitivity and low interference with excitation and emission filters set at 540/590 nm.

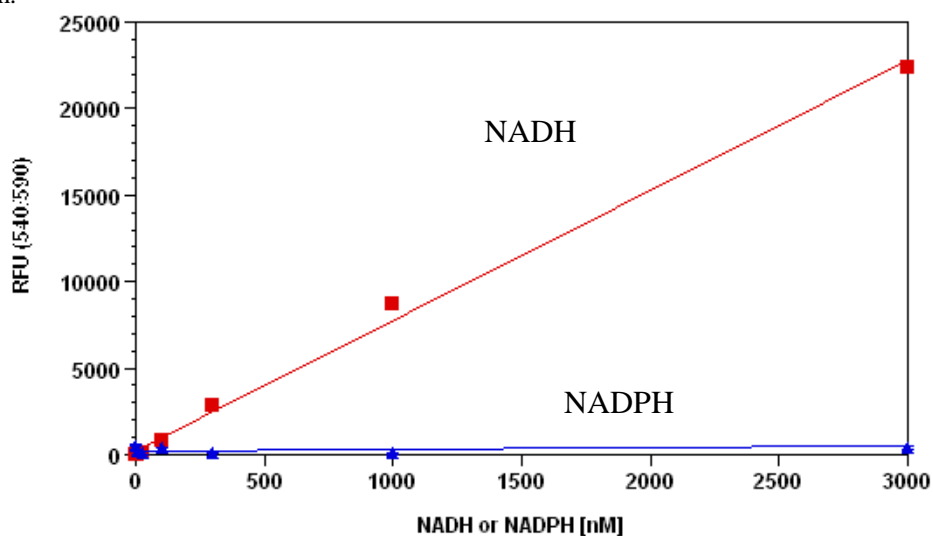


Figure 4 NADH dose response was measured on a 96-well black plate with Amplite™ NAD/NADH Assay Kit using a BMG LabTech NOVOSTar microplate reader. As low as 100 nM (10 pmol/well) of NADH was detected with 1 hour incubation time (n=3) while there was no response from NADPH.

Amplite™ Fluorimetric NAD/NADH Ratio Assay Kit *Red Fluorescence*

Quick Facts at a Glance

Product Number: 15263	Kit Size: 250 assays
Recording Modality:	Fluorescence setting at Ex/Em = 540/590 nm
Instrument Platform:	Any fluorescence microplate readers with proper filters

Kit Features

Nicotinamide adenine dinucleotide (NAD⁺) and nicotinamide adenine dinucleotide phosphate (NADP⁺) are two important cofactors found in cells. NADH is the reduced form of NAD⁺, and NAD⁺ is the oxidized form of NADH. It forms NADP with the addition of a phosphate group to the 2' position of the adenyl nucleotide through an ester linkage. NADP is used in anabolic biological reactions, such as fatty acid and nucleic acid synthesis, which require NADPH as a reducing agent. In chloroplasts, NADP is an oxidizing agent important in the preliminary reactions of photosynthesis. The NADPH produced by photosynthesis is then used as reducing power for the biosynthetic reactions in the Calvin cycle of photosynthesis. The traditional NAD/NADH and NADP/NADPH assays are carried out by monitoring the NADH or NADPH with absorption at 340 nm. This method suffers from low sensitivity and high interference since the assay is run in the UV range that requires expensive quartz microplate. This Amplite™ Fluorimetric NAD/NADH Ratio Assay Kit provides a convenient method for the sensitive detection of NAD, NADH and their ratio. The enzymes in the system specifically recognize NAD/NADH in an enzyme cycling reaction. There is no need to purify NAD/NADH from the sample mix. The enzyme cycling reaction significantly increases detection sensitivity. Meanwhile, the kit comes with NAD, NADH extraction solution and Lysis buffer, which makes it easy to use and be adapted to automation. In addition, this assay has very low background since it is run in the red visible range that significantly reduces the interference from the biological samples. The assay has demonstrated high sensitivity and low interference with excitation and emission filters set at 540/590 nm.

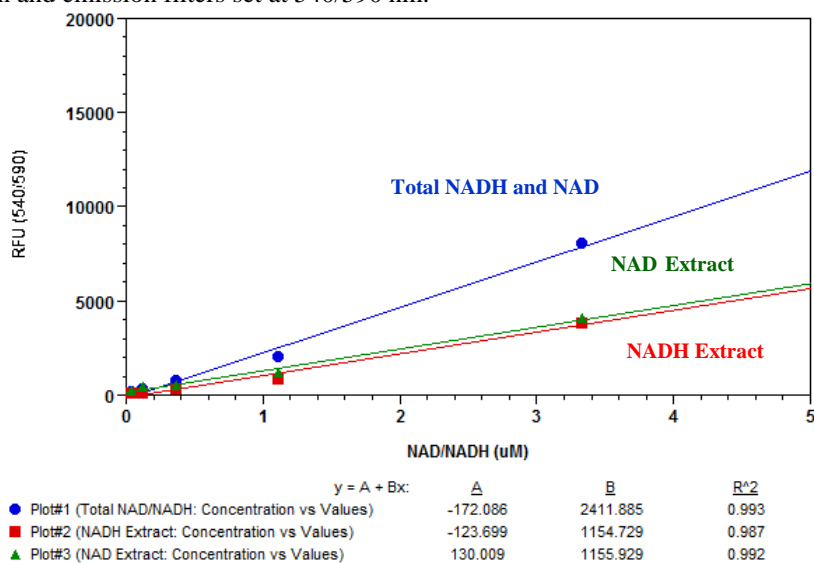


Figure 5 Total NADH and NAD, and their extract dose response were measured on a 96-well black with Amplite™ NAD/NADH Ratio Assay Kit in a 96-well black plate using a Gemini microplate reader (Molecular Devices). As low as 100 nM (10 pmol/well) of NADH was detected with 1 hour incubation time (n=3).

Amplite™ Fluorimetric NADPH Assay Kit *Red Fluorescence*

Quick Facts at a Glance

Product Number: 15262

Recording Modality:

Instrument Platform:

Kit Size: 400 assays

Fluorescence setting at Ex/Em = 540/590 nm

Any fluorescence microplate readers with proper filters

Kit Features:

Nicotinamide adenine dinucleotide (NAD⁺) and nicotinamide adenine dinucleotide phosphate (NADP⁺) are two important cofactors found in cells. NADH is the reduced form of NAD⁺, the oxidized form of NADH. It forms NADP with the addition of a phosphate group to the 2' position of the adenyl nucleotide through an ester linkage. NADP is used in anabolic biological reactions, such as fatty acid and nucleic acid synthesis, which requires NADPH as a reducing agent. In chloroplasts, NADP is an oxidizing agent important in the preliminary reactions of photosynthesis. The NADPH produced by photosynthesis is used as reducing power for the biosynthetic reactions in the Calvin cycle of photosynthesis. The traditional NAD/NADH and NADP/NADPH assays are carried out by monitoring the NADH or NADPH with absorption at 340 nm. This method suffers from low sensitivity and high interference since the assay is run in the UV range that requires expensive quartz microplate. This Amplite™ Fluorimetric NADPH Assay Kit provides a convenient method for the detection of NADPH. The enzymes in the system specifically recognize NADPH in an enzyme reducing reaction. In addition, this assay has very low background since it is run in the red visible range that significantly reduces the interference from the biological samples. The assay has demonstrated high sensitivity and low interference with excitation and emission filters set at 540/590 nm.

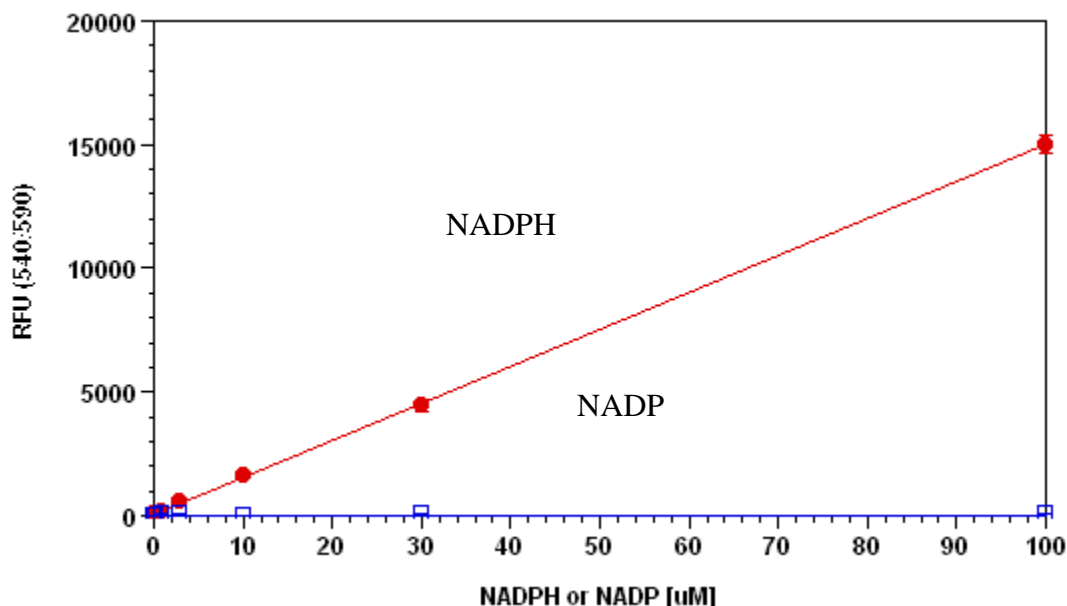


Figure 6 NADPH dose response was measured on a 96-well black plate with Amplite™ NADPH Assay Kit using a BMG LabTech NOVOSTar microplate reader. As low as 1 μ M (10 nmol/well) of NADPH was detected with 1 hour incubation time (n=3) while there was no response from NADP.

Amplite™ Fluorimetric NADP/NADPH Assay Kit *Red Fluorescence*

Quick Facts at a Glance

Product Number: 15259

Kit Size: 400 assays

Recording Modality:

Fluorescence setting at Ex/Em = 540/590 nm

Instrument Platform:

Any fluorescence microplate readers with proper filters

Kit Features

Nicotinamide adenine dinucleotide (NAD⁺) and nicotinamide adenine dinucleotide phosphate (NADP⁺) are two important cofactors found in cells. NADH is the reduced form of NAD⁺, and NAD⁺ is the oxidized form of NADH. It forms NADP with the addition of a phosphate group to the 2' position of the adenyl nucleotide through an ester linkage. NADP is used in anabolic biological reactions, such as fatty acid and nucleic acid synthesis, which require NADPH as a reducing agent. In chloroplasts, NADP is an oxidizing agent important in the preliminary reactions of photosynthesis. The NADPH produced by photosynthesis is then used as reducing power for the biosynthetic reactions in the Calvin cycle of photosynthesis. The traditional NAD/NADH and NADP/NADPH assays are carried out by monitoring the NADH or NADPH with absorption at 340 nm. This method suffers low sensitivity and high interference since the assay is run in the UV range that requires expensive quartz microplate. This Amplite™ NADP/NADPH Assay Kit provides a convenient method for the sensitive detection of NADP, NADPH and their ratio. The enzymes in the system specifically recognize NADP/NADPH in an enzyme cycling reaction. There is no need to purify NADP/NADPH from sample mix. The enzyme cycling reaction significantly increases detection sensitivity. In addition, this assay has very low background since it is run in the red visible range that significantly reduces the interference from biological samples. The assay has demonstrated high sensitivity and low interference with excitation and emission filters set at 540/590 nm.

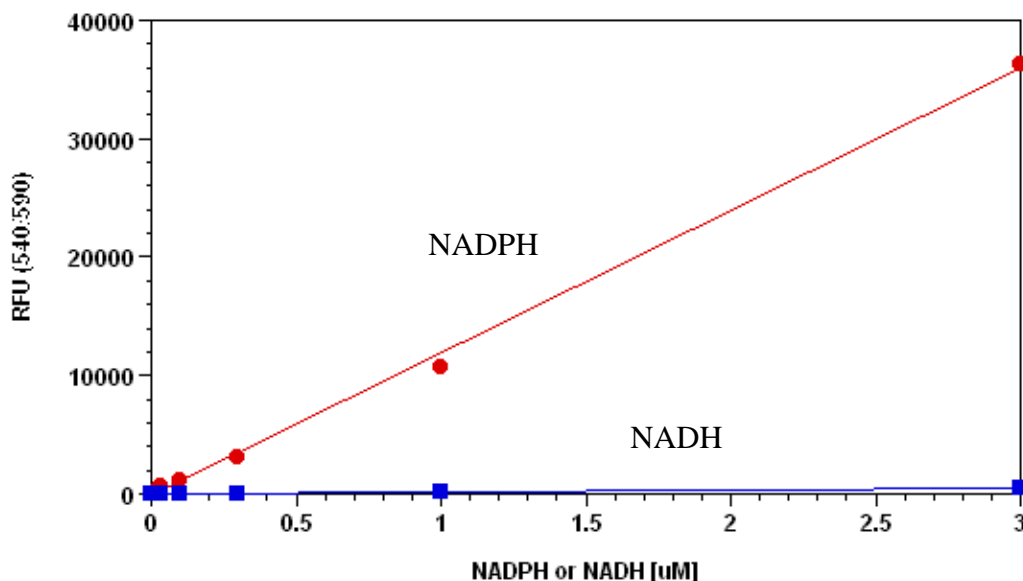


Figure 7 NADPH dose response was measured on a 96-well black plate with Amplite™ NADP/NADPH Assay Kit using a BMG LabTech NOVOSTar microplate reader. As low as 10 nM (1 pmol/well) of NADPH was detected with 30 min incubation time (n=3) while there was no response from NADH.

Amplite™ Fluorimetric NADP/NADPH Ratio Assay Kit *Red Fluorescence*

Quick Facts at a Glance

Product Number: 15264	Kit Size: 250 assays
Recording Modality:	Fluorescence setting at Ex/Em = 540/590 nm
Instrument Platform:	Any fluorescence microplate readers with proper filters

Kit Features

Nicotinamide adenine dinucleotide (NAD⁺) and nicotinamide adenine dinucleotide phosphate (NADP⁺) are two important cofactors found in cells. NADH is the reduced form of NAD⁺, and NAD⁺ is the oxidized form of NADH. It forms NADP with the addition of a phosphate group to the 2' position of the adenyl nucleotide through an ester linkage. NADP is used in anabolic biological reactions, such as fatty acid and nucleic acid synthesis, which require NADPH as a reducing agent. In chloroplasts, NADP is an oxidizing agent important in the preliminary reactions of photosynthesis. The NADPH produced by photosynthesis is then used as reducing power for the biosynthetic reactions in the Calvin cycle of photosynthesis. The traditional NAD/NADH and NADP/NADPH assays are carried out by monitoring the NADH or NADPH with absorption at 340 nm. This method suffers from low sensitivity and high interference since the assay is run in the UV range that requires expensive quartz microplate. This Amplite™ NADP/NADPH Ratio Assay Kit provides a convenient method for the sensitive detection of NADP, NADPH and their ratio. The enzymes in the system specifically recognize NADP/NADPH in an enzyme cycling reaction. There is no need to purify NADP/NADPH from sample mix. The enzyme cycling reaction significantly increases detection sensitivity. Meanwhile, the kit comes with NADP, NADPH extract solution and Lysis buffer which make it easy to use and be adapted to automation. In addition, this assay has very low background since it is run in the red visible range that significantly reduces the interference from biological samples. The assay has demonstrated high sensitivity and low interference with excitation and emission filters set at 540/590 nm.

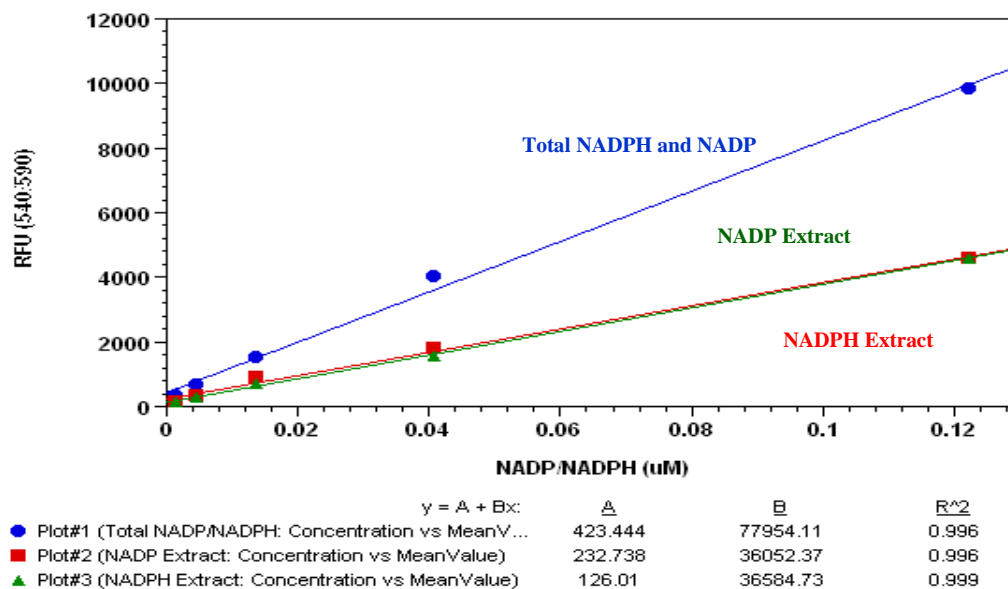


Figure 8 Total NADPH and NADP, and their extract dose response were measured on a 96-well black plate with Amplite™ NADP/NADPH Ratio Assay Kit using a Gemini microplate reader (Molecular Devices).