SZABO SCANDIC

Part of Europa Biosite

Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

Weitere Information auf den folgenden Seiten!
See the following pages for more information!

Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

linkedin.com/company/szaboscandic

Elite™ Fluorimetric NADP/NADPH Assay Kit (Red Fluorescence)

CATALOG NUMBER: CA-N418, 400 assays

Description

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. NAD 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 existing NADP/NADPH assays are run in UV range by absorption. The assays suffer low sensitivity and high interference. This Elite™ Fluorimetric NADP/NADPH Assay Kit provides a convenient method for sensitive detection of NADP and NADPH. The enzymes in the system specifically recognize NADP/NADPH in an enzyme cycling reaction that 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 resulted from biological samples. There is no need to purify NADP/NADPH from sample mix.

The Elite™ Fluorimetric NADP/NADPH Assay Kit provides a sensitive, one-step assay to detect as little as 1 picomoles of NADP(H) in a 100 µl assay volume (10 nM; Figure 1). The assay can be performed in a convenient 96-well or 384-well microtiter-plate format and readily adapted to automation. Its signal can be easily read by either a fluorescence microplate reader at Ex/Em = 540/590 nm or an absorbance microplate reader at ~576 nm. The longer red emission minimizes the interference from the autofluorescence of biological samples.

Features

- **Broad application**: NADP/NADPH detection in solution or cell extracts.
- **Sensitive**: Detect as low as 1 picomoles of NADP/NADPH in solution.
- **Continuous**: Easily adapted to automation without a separation step.
- **Convenient**: Formulated to have minimal hands-on time. No wash required.
- **Non-radioactive**: No special requirement for waste disposal.

Kit Components

- **Component A**: NADP/NADPH Recycling Enzyme Mixture, 2 bottles (lyophilized powder).
- **Component B**: NADPH Sensor Buffer, 1 bottle (20 ml).
- **Component C**: NADPH Standard (FW: 833.36), 1 vial (167 µg).
- **Component G**: NADP/NADPH Lysis Buffer, 1 bottle (10 ml).

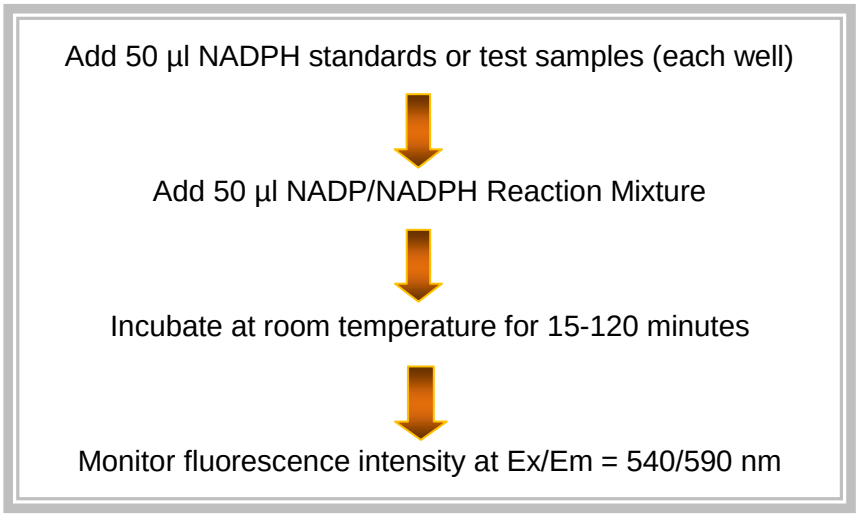
Storage

Keep **Component A** in freezer (-20 ^{o}C) and avoid exposure to light; **Component C** at -20 ^{o}C; **Component B &G** at 4 ^{o}C.

Tel: 1-800-919-0755 • Fax: 1-240-683-5852 • info@eEnzyme.com • www.eEnzyme.com

Assay Protocol (for 96-Well Plate)

Brief Summary of the Assay



1. **Prepare NADPH stock solution:**

   Add 200 µl of PBS buffer into the vial of NADPH standard (Component C) to have 1 mM (1 nmol/ µl) NADPH stock solution.
   Note: The unused NADPH stock solution should be divided into single use aliquots and stored at -20 ^{o}C.

2. **Prepare NADP/NADPH reaction mixture:**

   Add 10 ml of NADP/NADPH Sensor Buffer (Component B) to the bottle of NADP/NADPH Recycling Enzyme Mixture (Component A), and mix well.

   Note: This NADP/NADPH reaction mixture is enough for two 96-well plates or four 384-well plates. The unused NADP/NADPH reaction mixture should be divided into single use aliquots and stored at -20 ^{o}C.

3. **Prepare serially diluted NADPH standards (0 to 10 µM):**

   3.1. Add 10 µl of NADPH stock solution (from Step 1) into 990 µl PBS buffer (pH 7.4) to generate 10 µM (10 pmols/ µl) NADPH standard solution.

   Note: Diluted NADPH standard solution is unstable, and should be used within 4 hours.

   3.2. Take 200 µl of 10 µM NADPH standard solution to perform 1:3 serial dilutions to get 3, 1, 0.3, 0.1, 0.03, 0.01, 0.003, and 0 µM serially dilutions NADPH standards.

   3.3. Add serially diluted NADPH standards and NADP/NADPH containing test samples into a solid black 96-well microplate as described in Tables 1 and 2.

   Note: Prepare cells or tissue samples as desired or as described in appendix A. NADP/NADPH Lysis Buffer (Component G) can be used for lysing the cells.

Table 1. Layout of NADPH standards and test samples in a solid black 96-well microplate

BL	BL	TS	TS								
NS1	NS1										
NS2	NS2										
NS3	NS3										
NS4	NS4										

Tel: 1-800-919-0755 • Fax: 1-240-683-5852 • info@eEnzyme.com • www.eEnzyme.com

NS5	NS5										
NS6	NS6										
NS7	NS7										

Note: NS= NADPH Standards; BL=Blank Control; TS=Test Samples.

Table 2. Reagent composition for each well

NADPH Standard	Blank Control	Test Sample
Serial Dilutions: 50 µl	PBS: 50 µl	50 µl

Note: Add the serially diluted NADPH standards from 0.003 µM to 3 µM into wells from NS1 to NS7 in duplicate. High concentration of NADPH (e.g., >100 µM, final concentration) may cause reduced fluorescence signal due to the over oxidation of NADPH sensor (to a non-fluorescent product).

4. Run NADP/NADPH assay in supernatants reaction:

4.1. Add 50 µl of NADPH reaction mixture (from Step 2) into each well of NADPH standard, blank control, and test samples (from Step 3.3) to make the total NADPH assay volume of 100 µl/well.

4.2. Incubate the reaction at room temperature for 15 minutes to 2 hours, protected from light.

4.3. Monitor the fluorescence increase with a fluorescence plate reader at Ex/Em = 540/590 nm.

Note: The contents of the plate can also be transferred to a white clear bottom plate and read by an absorbance microplate reader at the wavelength of 576 ± 5 nm. The absorption detection has lower sensitivity compared to fluorescence reading.

Data Analysis

The fluorescence in blank wells (with the PBS buffer only) is used as a control, and is subtracted from the values for those wells with the NADPH reactions. A NADPH standard curve is shown in Figure 1.

Note: The fluorescence background increases with time, thus it is important to subtract the fluorescence intensity value of the blank wells for each data point.

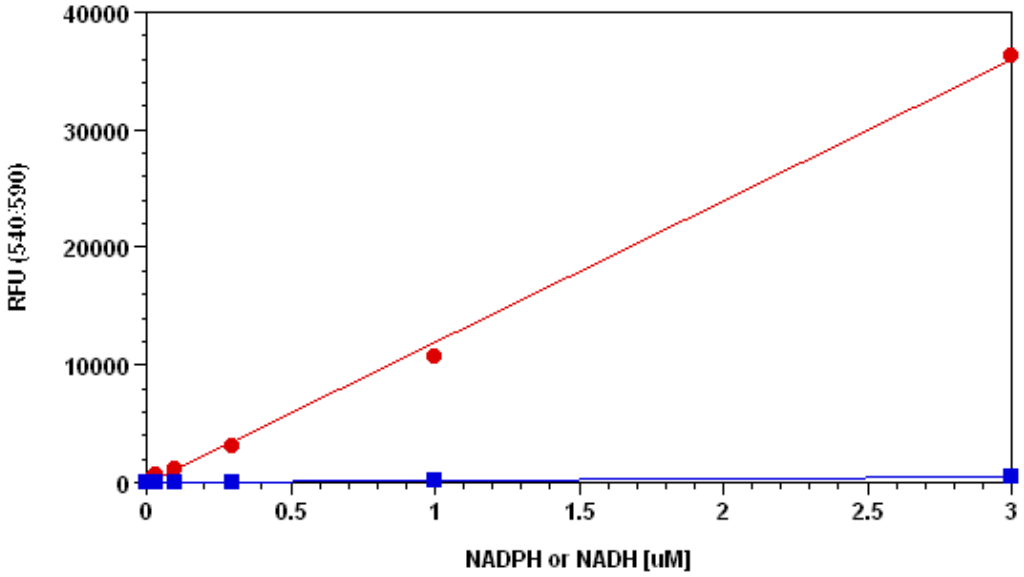


Figure 1. NADPH dose response was measured with Elite™ NADP/NADPH Assay Kit in a 96-well black plate using a NOVOStar microplate reader (BMG Labtech). As low as 10 nM (1 pmol/well) of NADPH can be detected with 1 hour incubation (n=3) while there is no response from NADH.

Tel: 1-800-919-0755 • Fax: 1-240-683-5852 • info@eEnzyme.com • www.eEnzyme.com

Appendix A

Sample Lysis Preparation for NADH/NAD or NADPH/NADP Assay

1. Lysis of bacterial cells

   Collecting bacterial cells by centrifugation ((10,000 x g, 0°C, 15 min). Use about 100 to 10 million cells/mL lysis buffer, leave at room temperature for 15 minutes. And then centrifuge at 2500 rpm for 5 minutes, use the supernatant for the assay.

2. Lysis of plant cells:

   Homogenize the leave with the lysis buffer at 200 mg/mL, and then centrifuge at 2500 rpm for 5-10 minutes, use the supernatant for the assay.

3. Lysis of mammalian cells

   Simply remove medium from the plates (wells), use about 100 uL lysis buffer per 1-5 million cells (or 100uL/ well in a 96-well cell culture plate), and leave at room temperature for 15 minutes. You can use the cell lysate directly or simply centrifuge at 1500 rpm for 5 minutes, use the supernatant for the assay.

4. Lysis of tissues.

   Weight ~20 mg tissue, wash with cold PBS, homogenize with 400 µl of lysis buffer in a micro-centrifuge tube, and then centrifuge at 2500 rpm for 5-10 minutes, use the supernatant for the assay.

   Note: Lysis buffer is supplied as Component G (Cat# CA-N015g).