NADP/NADPH Assay Kit (BA0238)

Product Datasheet | SKU BA0238

Description

A fluorimetric assay kit for the quantitative determination of NADP+, NADPH and their ratio in cell and tissue extracts. The kit uses a glucose dehydrogenase cycling reaction that generates a highly fluorescent product measured at λex/em = 530/585 nm, with a detection limit of 0.01 µM and linearity up to 1 µM in a 96-well plate format.

Specifications

Detection Method	Fluorimetric (FL530/585 nm)
Detection Range	0.01 µM to 1 µM NADP+/NADPH (96-well plate)
Sample Type	Cell extracts,Tissue extracts
Species Reactivity	All
Assay Time	30 min
Kit Size	100 Assays
Equipment Required	Microplate reader
Storage	-20°C
Shelf Life	12 months
Shipping	Gel Pack

Kit Components

Component	Quantity	Storage
Assay Buffer	10 mL	-20°C
Enzyme A	120 µL	-20°C
G6P	120 µL	-20°C
Enzyme B	120 µL	-20°C
Probe	750 µL	-20°C
NADP Standard	0.5 mL	-20°C
NADP Extraction Buffer	12 mL	-20°C
NADPH Extraction Buffer	12 mL	-20°C

Ordering Information

Assay Genie Cat. No.	Pack Size
BA0238	100 Assays

Assay Procedure

Note: This is a sample protocol. Protocols are batch/lot specific - always follow the instructions supplied with your kit.

1. Sample Preparation. For tissues, weigh ~20 mg tissue per sample and wash with cold PBS. For cells, wash with cold PBS and pellet ~10^5 cells per sample. Homogenise samples in a 1.5 mL tube with either 100 µL NADP+ extraction buffer (for NADP+) or 100 µL NADPH extraction buffer (for NADPH). Heat extracts at 60°C for 5 min, then add 20 µL Assay Buffer and 100 µL of the opposite extraction buffer to neutralise. Briefly vortex and spin down at 14,000 rpm for 5 min; use the supernatant for the assay. Determination of both NADP+ and NADPH requires extractions from two separate samples.

2. Calibration Curve. Prepare 5000 µL of 1 µM NADP+ Premix by mixing 5 µL of 1 mM Standard with 4995 µL distilled water. Dilute the standard to give 1.0, 0.6, 0.3 and 0 µM points (see Standard Dilution table). Transfer 50 µL standards into wells of a black flat-bottom 96-well plate.
3. Samples. Add 50 µL sample per well in separate wells.
4. Reagent Preparation. For each reaction well, prepare Working Reagent by mixing 45 µL Assay Buffer, 1 µL Enzyme A, 1 µL Enzyme B, 1 µL G6P and 5 µL Probe. Fresh reconstitution is recommended.
5. Reaction. Add 50 µL Working Reagent per well quickly. Tap plate to mix.
6. Read fluorescence at λex/em = 530/585 nm for time zero (F0) and again (F30) after a 30-min incubation at room temperature. Protect the plate from light during incubation.

Standard Dilution (NADP+)

No	Premix + H2O	NADP (µM)
1	100 µL + 0 µL	1.0
2	60 µL + 40 µL	0.6
3	30 µL + 70 µL	0.3
4	0 µL + 100 µL	0

Calculation

Compute the ΔF for each standard and sample by subtracting F0 from F30. Plot the standard ΔF values and determine the slope. [NADP(H)] = (ΔF_SAMPLE – ΔF_BLANK) / Slope (µM^-1) × n (µM), where ΔF_SAMPLE and ΔF_BLANK are the changes in fluorescence intensity of the Sample and Blank (STD 4) respectively, Slope is the slope of the standard curve and n is the dilution factor. If sample ΔF values exceed that of the 1 µM standard, dilute the sample in distilled water and repeat, multiplying the result by the dilution factor.

Notes

- At these concentrations, the standard curves for NADP+ and NADPH are identical. Since NADPH in solution is unstable, only NADP is provided as the standard.
- This assay is based on an enzyme-catalysed kinetic reaction. Addition of Working Reagent should be quick and mixing brief but thorough; use of a multi-channel pipettor is recommended.
- The following substances interfere and should be avoided in sample preparation: EDTA (>0.5 mM), ascorbic acid, SDS (>0.2%), sodium azide, NP-40 (>1%) and Tween-20 (>1%).
- Reagents are for research use only. Refer to the MSDS for detailed safety information.