NADP/NADPH Assay Kit (BA0232)

Product Datasheet | SKU BA0232

Description

An ultrasensitive colorimetric assay for NADP+/NADPH based on a glucose dehydrogenase cycling reaction in which the formed NADPH reduces a formazan (MTT) reagent. The intensity of the reduced product colour at 565 nm is proportional to the NADP+/NADPH concentration in the sample and is not interfered with by NAD+/NADH.

Specifications

Detection Method	Colorimetric determination at 565 nm (520-600 nm)
Detection Range	Detection limit 0.1 µM, linearity up to 10 µM NADP+/NADPH in a 96-well plate assay
Sample Type	Cell or tissue extracts
Species Reactivity	All
Assay Time	30 minutes
Kit Size	100 Assays
Equipment Required	Microplate reader
Storage	The kit is shipped on ice. Store all reagents at -20°C.
Shelf Life	6 months after receipt.
Shipping	Gel Pack

Kit Components

Component	Quantity	Storage
Assay Buffer	10 mL	-20°C
Enzyme A	120 µL	-20°C
MTT Solution	1.5 mL	-20°C
Enzyme B	120 µL	-20°C
G6P	120 µL	-20°C
NADP Standard (1 mM)	0.5 mL	-20°C
NAD(P)/NAD(P)H Extraction Buffers	each 12 mL	-20°C

Ordering Information

Assay Genie Cat. No.	Pack Size
BA0232	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 per sample and wash with cold PBS; for cells wash with cold PBS and pellet ~10^5 cells. Homogenise in a 1.5 mL tube with 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. Vortex, spin down at 14,000 rpm for 5 min and use supernatant. Determination of both NADP and NADPH requires extractions from two separate samples.

2. Calibration curve: prepare 500 µL 10 µM NADP Premix by mixing 5 µL 1 mM Standard and 495 µL distilled water. Dilute standards as per the table and transfer 40 µL standards into wells of a clear-bottom 96-well plate.
3. Samples: add 40 µL sample per well in separate wells.
4. Reagent preparation: allow Enzyme to reach room temperature (15-30 min). For each reaction well prepare Working Reagent by mixing 80 µL Assay Buffer, 1 µL Enzyme A, 1 µL Enzyme B, 1 µL G6P and 14 µL MTT. Fresh reconstitution is recommended.
5. Reaction: add 80 µL Working Reagent per well quickly. Tap plate to mix briefly and thoroughly.
6. Read optical density (OD0) at time zero at 565 nm (520-600 nm) and OD30 after a 30-min incubation at room temperature.

Calibration Curve

No.	Premix	H2O Vol (µL)	[NADP] (µM)
1	100 µL	0 µL	10
2	80 µL	20 µL	8
3	60 µL	40 µL	6
4	40 µL	60 µL	4
5	30 µL	70 µL	3
6	20 µL	80 µL	2
7	10 µL	90 µL	1
8	0 µL	100 µL	0

Calculation

Subtract OD0 from OD30 for the standard and sample wells. Use the ΔOD values to determine sample concentration from the standard curve: [NADP(H)] = [(ΔOD_Sample – ΔOD_Blank) / Slope (μM^{-1})] × n (µM). If sample ΔOD values are higher than that of the 10 µM standard, dilute sample in distilled water and repeat, multiplying 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.
- 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.