Amplite® Colorimetric Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) Assay Kit

Catalog number: 11321
Unit size: 100 Tests

Component	Storage	Amount (Cat No. 11321)
Component A: GAPDH Probe	Freeze (< -15 °C), Minimize light exposure	1 mL
Component B: GAPDH Assay Buffer	Freeze (< -15 °C)	4 mL
Component C: GAPDH Substrate	Freeze (< -15 °C)	1 Bottle
Component D: NADH Standard	Freeze (< -15 °C), Minimize light exposure	1 Vial
Component E: GAPDH Positive Control	Freeze (< -15 °C)	1 Vial

OVERVIEW

The Amplite® Colorimetric GAPDH Activity Assay Kit is a sensitive and simple tool for monitoring GAPDH activity based on a coupled enzymatic reaction catalyzed by GAPDH. This reaction results in the formation of a colorimetric product with an absorbance at 450nm, which is directly proportional to the enzymatic activity of GAPDH present in cell culture or tissue samples. One unit (U) is the amount of enzyme that catalyzes the reaction of 1 µmol of substrate per minute. GAPDH (Glyceraldehyde-3-Phosphate Dehydrogenase) is a multifunctional protein that serves both as a glycolytic enzyme and as a uracil DNA glycosylase. In glycolysis, it catalyzes the conversion of glyceraldehyde-3-phosphate to 1,3-bisphosphate glycerate. GAPDH is also involved in many cellular processes such as apoptosis, membrane trafficking, glucose and iron metabolism, and nuclear translocation. The expression of GAPDH in cells is constitutive, making it a housekeeping gene. Dysregulation of GAPDH activity has been associated with carcinogenesis, abnormal cell growth, and late-onset Alzheimer's disease. Quantification of GAPDH expression levels in different experimental conditions or disease states can provide insights into metabolic changes associated with cellular processes such as proliferation, differentiation, and response to stress.

AT A GLANCE

Important Note

Thaw all the kit components at room temperature before starting the experiment.

Protocol Summary

1. Prepare the test samples, GAPDH Positive Control, and the serially diluted NADH standards (50 µL).
2. Add the GAPDH working solution (50 µL).
3. Incubate at room temperature for 10-30 minutes.
4. Measure the absorbance at 450 nm.

KEY PARAMETERS

Absorbance microplate reader

Absorbance	450 nm
Recommended plate	Clear bottom

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles

NADH Standard Stock Solution

1. Add 200 µL of PBS buffer to the vial of NADH standard (Component D) to make a 2 mM (2 nmol/µL) NADH stock solution. Store the solution at -80°C. Avoid repeated freeze/thaw cycles.

GAPDH Positive Control Stock Solution

1. To prepare a GAPDH stock solution, reconstitute the GAPDH Positive Control (Component E) by adding 40 µL of ddH2O. Mix well by pipetting and store at –20 °C.

   Note: Must be used within 2 months of reconstitution.

50X GAPDH Substrate Stock Solution

1. To prepare a 50X GAPDH Substrate stock solution, add 100 µL of ddH2O to the vial containing the GAPDH Substrate (Component C). After mixing, store the solution at -20°C. Avoid repeated freezing and thawing.

PREPARATION OF STANDARD SOLUTIONS

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/11321

NADH Standard
Add 3 µL of 2 mM NADH standard solution to 297 µL of PBS + 0.1% BSA Buffer to prepare a 200 µM NADH solution (STD7). Then, take 150 µL of STD7 and perform 1:2 serial dilutions with PBS + 0.1% BSA Buffer to create a series of NADH standards from STD7 to STD1.

PREPARATION OF WORKING SOLUTION

GAPDH Working Solution

1. Add 1 mL of the GAPDH Probe (Component B) to the bottle containing 4 mL of the GAPDH Assay Buffer (Component A), and mix well.
2. Add 50 µL of the GAPDH Substrate stock solution to the same bottle and mix well.

   Note: This GAPDH working solution should be freshly prepared before each experiment and protected from light. A 5 mL solution is enough for 100 tests. Please prepare the necessary amount of GAPDH working solution based on this proportion.

SAMPLE EXPERIMENTAL PROTOCOL

GAPDH Positive Control

1. Prepare one or more GAPDH positive control samples along with the test sample. The recommended concentration for the GAPDH

positive control is 50 mU/mL in PBS + 0.1% BSA.

Table 1. Layout of GAPDH standards and test samples in a 96-well clear bottom microplate. (STD = NADH Standards (STD1-STD7, 3.125 to 200 uM), BL= Blank Control, TS = Test Samples.)

BL	BL	Positive Control	TS
STD 1	STD 1	...	...
STD 2	STD 2	...	...
STD 3	STD 3		
STD 4	STD 4		
STD 5	STD 5		
STD 6	STD 6		
STD 7	STD 7		

Table 2. Reagent composition for each well.

Well	Volume	Reagent
STD 1 -STD 7	50 µL	NADH Serial Dilutions (3.125 to 200 µM)
BL	50 µL	PBS
GAPDH Positive Control	50 µL	GAPDH Positive Control
TS	50 µL	Test Sample

1. Prepare the NADH standards (STD1-7), blank controls (BL), GAPDH Positive Control, and test samples (TS) according to the layout provided in Tables 1 and 2. When using a 384-well plate, use 25 µL of reagent per well instead of 50 µL.

2. Add 50 µL of GAPDH Working Solution to each well containing the NADH standard, blank control, GAPDH Positive Control, and test samples. For a 384-well plate, add 25 µL of GAPDH Working Solution to each well instead.

3. Incubate at room temperature for 10–30 minutes, protected from light.

4. Monitor the absorbance intensity with an absorbance microplate reader at 450 nm.

EXAMPLE DATA ANALYSIS AND FIGURES

The absorbance reading in blank wells (containing only PBS) is used as a control and subtracted from the readings of wells with NADH standards, GAPDH positive controls, and test samples. Figure 1 shows the standard curve for NADPH. To calculate the NADH concentrations of the samples using the standard curve, we recommend using the Online Linear Regression Calculator, available at:

https://www.aatbio.com/tools/linear-logarithmic-semi-log-regression-online-calculator

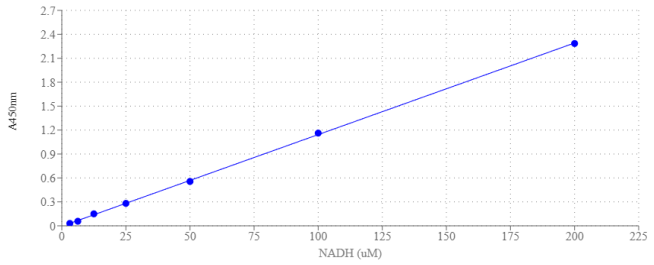


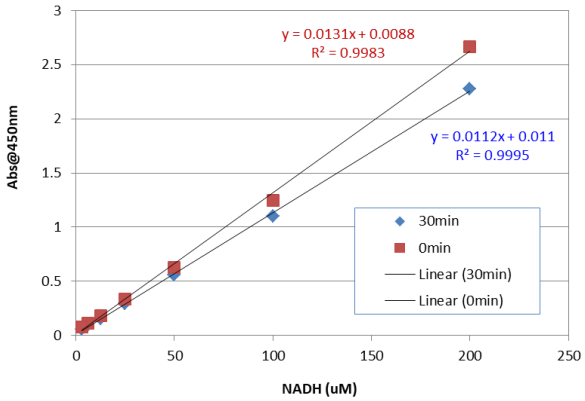
Figure 1. The NADH dose response was measured using the Amplite® Colorimetric Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) Assay Kit on a 96-well clear bottom microplate. The assay was incubated for 10 minutes and measured at 450 nm using a ClarioStar microplate reader (BMG).

Data Analysis Example

Calculate GAPDH Activity Example

- GAPDH positive control: 50 mU/mL in PBS+0.1% BSA
- 0 min to 10 min kinetic reading,
- Use 0 min and 10 min absorbance readings to do the calculation.

1. Plot NADH Calibration curve at 0min and 10min.



2. Calculate NADH generated during 10 minutes.

Time	GAPDH Positive Control	NADPH (µM)
0 min	0.245	18.11
30 min	2.45	217.46

GADPH Positive Control	NADPH generated in 30 min	µM/min=mU/mL
50 mU/mL	200 µM	6.7

Note:

- 1 unit (U) is the amount of enzyme that catalyzes the reaction of 1 µmol of substrate per minute
- nmole/min/mL= uM/min = mU/mL

DISCLAIMER

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