Technical Bulletin

GAPDH Activity Assay Kit

Catalogue number MAK277

Product Description

GAPDH (Glyceraldehyde-3-Phosphate Dehydrogenase, EC 1.2.1.12) catalyzes the conversion of glyceraldehyde-3-phosphate (GAP) to 1,3-bisphosphate glycerate (BPG) and plays a key role in glycolysis. The enzyme is involved in cellular processes such as apoptosis, membrane trafficking, iron metabolism, and nuclear translocation. GAPDH (housekeeping gene) expression is stable and constitutive. Deregulation of GAPDH activity is associated with abnormal cell proliferation and carcinogenesis. Accurate quantitation of GAPDH activity is important for diagnosing diseases and studying normal cellular physiology.

The GAPDH Activity Assay Kit provides a simple and sensitive method for monitoring GAPDH activity in various samples. GAPDH activity is determined in a coupled enzyme reaction in which GAP is converted to BPG by GAPDH. This results in a colorimetric (450 nm) product proportional to the enzymatic activity present.

The assay is sensitive to 100 mUnits/mL. One unit of GAPDH activity is the amount of enzyme that will generate 1.0 mmole of NADH per minute at pH 7.2 at 37 °C.

The assay kit is suitable for use with various tissue and cell culture samples.

Components

The kit is sufficient for 100 assays in 96 well plates.

- GAPDH Assay Buffer — 25 mL
  Catalog Number MAK277A
- GAPDH Substrate — 200 µL
  Catalog Number MAK277B
- GAPDH Developer — 1 vial
  Catalog Number MAK277C
- NADH Standard (500 nmole) — 1 vial
  Catalog Number MAK277D
- GAPDH Positive Control — 1 vial
  Catalog Number MAK277E

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (for example., multichannel pipettor)
- Multiwell plate reader
- Clear flat-bottom 96-well plates for colorimetric assay

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Storage at –20 °C, protected from light, is recommended.

Preparation Instructions

Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

GAPDH Assay Buffer – Allow buffer to come to room temperature before use. Store at –20 °C.

GAPDH Substrate – Ready to use as supplied. Keep on ice while in use. Divide into aliquots and store at -20 °C. Use within 2 months.

GAPDH Developer - Reconstitute with 220 µL of water. Mix well by pipetting (do not vortex). Store at –20 °C. Use within 2 months of reconstitution.

NADH Standard – Reconstitute with 400 µL of water to generate a 1.25 mM (1.25 nmole/µL) standard solution. Mix well, then aliquot and store at –20 °C. Keep on ice while in use. Use within 2 months of reconstitution.

GAPDH Positive Control - Reconstitute with 100 µL of water. Mix thoroughly, then aliquot and store at –70 °C. Keep on ice while in use. Use within 2 months of reconstitution.

Procedure

All Samples and Standards should be run in duplicate.

GAPDH Standards for Colorimetric Detection

Add 0, 2, 4, 6, 8, and 10 µL of the 1.25 mM NADH Standard solution into a 96 well plate, generating 0 (blank), 2.5, 5.0, 7.5, 10, and 12.5 nmole/well Standards. Add GAPDH Assay Buffer to each well to bring the volume to 50 µL.

The assay requires 50 µL of Sample for each reaction(well).

Whole cells (1 x 10^6) or tissues (~10 mg) can be homogenized with 100 µL of ice-cold GAPDH Assay Buffer and kept on ice for 10 minutes. Centrifuge the Samples at 10,000 x g for 5 minutes at 4 °C to remove insoluble material. The supernatant can be used for the GAPDH assay.

Add 1–50 µL Samples into wells of a 96 well plate. Bring Samples to a final volume of 50 µL with GAPDH Assay Buffer.

Add 2–20 µL of GAPDH Positive Control into wells and bring Samples to a final volume of 50 µL with GAPDH Assay Buffer.

Notes: For unknown samples, it is suggested to test several Sample volumes to make sure the readings are within the range of the Standard curve.

To control for background activity, a Sample blank may be Set up for each Sample by omitting the substrate (Table 1). The blank readings can be subtracted from the Sample readings.

Assay Reaction

1. Set up the Master Reaction Mix according to the scheme in Table 1. 50 µL of the Master Reaction Mix is required for each reaction (well).

Table 1.

Master Reaction Mix

Reagent	Samples and Standards	Sample Blank
GAPDH Assay Buffer	46 µL	48 µL
GAPDH Developer	2 µL	2 µL
GAPDH Substrate	2 µL	-

2. Add 50 µL of the Master Reaction Mix to each Sample and Standard control well. Add 50 µL of the Sample Blank Mix to each Sample blank well. Mix well using a horizontal shaker or by pipetting.
3. For colorimetric assays, measure the absorbance at 450 nm (A450) in kinetic mode for 10–60 minutes at 37 °C.

Note: Incubation time depends on the GAPDH activity in the Samples. Measuring the OD in a kinetic mode and choosing two time points (T1 & T2) in the linear range to calculate the GAPDH activity of the Samples is recommended. The NADH Standard curve can be read in end point mode (i.e., at the end of Sample incubation time).

Results

Calculations

The reagent background for the assay is the value obtained for the 0 (assay blank) NADH Standard. Correct for the background by subtracting the 0 (assay blank) value from all readings. Background values can be significant and must be subtracted from all readings. Use the values obtained from the appropriate NADH standards to plot a standard curve.

Note: A new standard curve must be set up each time the assay is run.

The GAPDH activity of a sample may be determined by the following equation:

$$\text{GAPDH Activity (milliunit/mL)} = \frac{B \times \text{Sample Dilution Factor}}{T \times V}$$

B = Amount (nmole) of NADH generated
T = Time reaction incubated in minutes
V = Pretreated sample volume (mL) added to well
GAPDH activity is reported as nmole/min/mL = milliunit/mL. One unit of GAPDH is the amount of

enzyme that will generate 1.0 mmole of NADH per minute at pH 7.2 at 37 °C.

Example:

NADH (B) = 5.84 nmole
T = 45 minutes
Sample volume (V) = 0.05 mL
Sample dilution is 1

GAPDH activity is:

$$\frac{5.84 \times 1}{45 \times 0.05} = 2.59 \text{ milliunits/mL}$$

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For fluorescence assays, use black plates with clear bottoms. For colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Master Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Master Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

The information in this document is subject to change without notice and should not be construed as a commitment by the manufacturing or selling entity, or an affiliate. We assume no responsibility for any errors that may appear in this document.

Technical Assistance

Visit the tech service page at SigmaAldrich.com/techservice.

Standard Warranty

The applicable warranty for the products listed in this publication may be found at SigmaAldrich.com/terms.

Contact Information

For the location of the office nearest you, go to SigmaAldrich.com/offices.

Document # Rev 05/23