Gentaur Europe BVBA Voortstraat 49, 1910 Kampenhout BELGIUM
Tel 0032 16 58 90 45 info@gentaur.com

Human GAPDH Activity Assay Kit II

(Catalog # K2047-100; 100 assays; Store at -20°C)

I. Introduction:

Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH; 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 is a stable metabolic enzyme and is constitutively expressed. Therefore, it is considered as a housekeeping gene. Deregulation of GAPDH activity is associated with cell proliferation and carcinogenesis. Accurate detection of GAPDH activity is important for diagnosing diseases and studying normal cellular physiology. **BioVision's Human GAPDH Activity Assay Kit II** provides a simple and sensitive method for monitoring GAPDH activity in various sample types. In this assay, GAPDH catalyzes GAP into BPG and an intermediate, which then reacts with the developer to form a colored product that is measured at 450 nm. Our high-throughput adaptable assay can detect GAPDH activity as low as 10 mU/mL in a variety of samples.

$$\text{GAP + Phosphate + NAD}^+ \xrightarrow{\textbf{GAPDH}} \text{BPG + Intermediate} \xrightarrow{\textbf{Developer}} \text{Colored product (OD 450 nm)}$$

II. Applications:

- Measurement of GAPDH activity in various tissues and cells
- Analysis of glycolysis and pentose phosphate pathways

III. Sample Types:

- Animal tissues: Liver, Heart etc.
- Cell culture: Adherent or suspension cells.

IV. Kit Contents:

Components	K2047-100	Cap Code	Part Number
GAPDH Assay Buffer	25 ml	WM	K2047-100-1
GAPDH Substrate	1 vial	Blue	K2047-100-2
GAPDH Developer	1 vial	Red	K2047-100-3
NADH Standard	1 vial	Yellow	K2047-100-4
Human GAPDH Positive Control	1 vial	Amber	K2047-100-5
GAPDH Reconstitution Buffer	1.5 ml	White	K2047-100-6

V. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer (ELISA reader)

VI. Storage and Reagent Preparation:

Store the kit at -20°C, protected from light. Read the entire protocol before performing the assay.

- **GAPDH Assay Buffer & GAPDH Reconstitution Buffer:** Warm to room temperature (RT) before use. Store at -20°C.
- **GAPDH Substrate:** Reconstitute the vial with 220 µl of GAPDH Assay Buffer. Pipette up and down to dissolve completely. Keep on ice while in use. Divide into aliquots and store at -20°C. Use within two months.
- **GAPDH Developer:** Reconstitute the vial with 220 µl ddH_2O. Pipette up and down to dissolve completely. Store at -20°C. Use within two months.
- **NADH Standard:** Reconstitute the vial with 400 µl ddH_2O to generate 1.25 mM (1.25 nmol/µl) NADH stock Standard solution. Keep on ice while in use. Divide into aliquots and store at -20°C. Use within two months.
- **Human GAPDH Positive Control:** Reconstitute the vial with 100 µl GAPDH Reconstitution Buffer. Vortex several times and put on ice for 5 min to completely dissolve. Divide into aliquots and store at -20°C. Avoid freeze/thaw cycles. Keep on ice while in use. Use within two months.

VII. GAPDH Activity Assay Protocol:

1. Sample Preparation: For whole cells or tissue lysate, rapidly homogenize tissue (~10 mg) or cells (1 x 10^6) with 100 µl GAPDH Assay Buffer and keep on ice for 10 min. Centrifuge at 10,000 x g for 5 min and collect the supernatant. Dilute the cell lysates 50 fold by adding 10 µl of cell lysates to 490 µl of GAPDH Assay Buffer and mix well. Add 1-50 µl of diluted sample(s) per well. Adjust the volume to 50 µl/well with GAPDH Assay Buffer. For **GAPDH Positive Control**, dilute the reconstituted Human GAPDH Positive Control 10 fold by adding 20 µl of the reconstituted Human GAPDH to 180 µl of GAPDH Reconstitution Buffer. Add 1-20 µl of diluted Human GAPDH Positive Control into respective wells and adjust the volume to 50 µl/well with GAPDH Assay Buffer.

Notes:

a. For Unknown Samples, we suggest testing several doses to ensure the readings are within the Standard Curve range.
b. For samples having background, prepare a parallel sample well labeled as **Sample Background Control**.

2. NADH Standard Curve Preparation: Add 0, 2, 4, 6, 8 and 10 µl of 1.25 mM NADH stock Standard into a series of wells in 96 well clear plate to generate 0, 2.5, 5.0, 7.5, 10 and 12.5 nmol/well of NADH Standard. Adjust the volume to 50 µl/well with GAPDH Assay Buffer.

3. Reaction Mix Preparation: Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µl Mix containing:

	Reaction Mix	Background Control Mix*
GAPDH Assay Buffer	46 µl	48 µl
GAPDH Developer	2 µl	2 µl
GAPDH Substrate	2 µl	----

Add 50 µl of Reaction Mix to each well containing Standard, Positive Control and sample(s). *For samples having high background, add 50 µl of Background Control Mix to the Sample Background Control well. Mix well.

4. Measurement: Measure the plate at 450 nm in kinetic mode for 10-60 min. at 37°C.

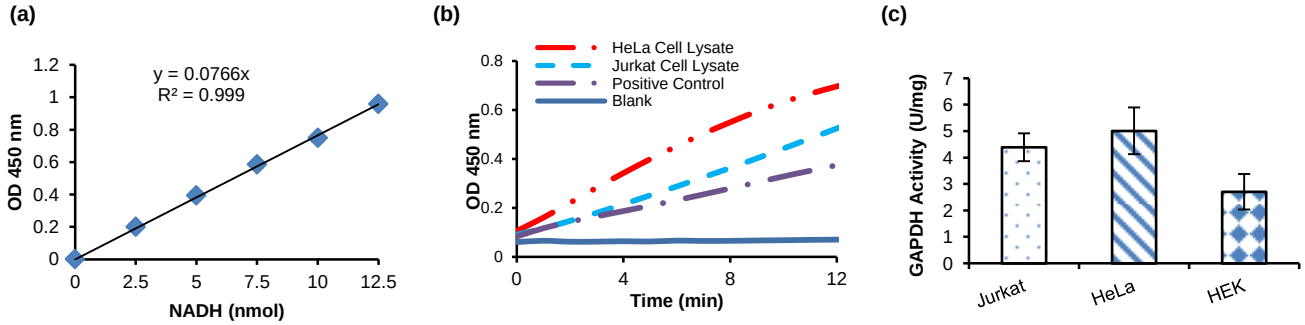
Note: Incubation time depends on the GAPDH activity in the samples. We recommend measuring the OD in a kinetic mode and choosing any two time points (T_1 & T_2) in the linear range to calculate the GAPDH activity of the samples. The NADH Standard Curve can be read in End point mode (i.e. at the end of sample incubation time).

5. Calculation: Subtract the 0 Standard reading from all Standard readings and Sample Background Control reading from all sample readings respectively. Plot the NADH Standard Curve. Choose any two time points within the linear portion of the curve (T_1 & T_2) for each sample type. Subtract the Sample Background Control readings from the corresponding sample readings for the chosen T_1 & T_2 time points. Apply the corrected sample readings to the NADH Standard Curve to get B nmol of NADH generated during the reaction time ($\Delta T = T_2 - T_1$).

Sample GAPDH Activity = B/(ΔT X V) x D = nmol/min/µl = mU/µl = U/ml

Where: **B** = NADH amount from the Standard Curve (nmol)
ΔT = Reaction time (min)
V = Sample volume used (µl)
D = Sample dilution factor

Unit Definition: One unit of GAPDH is the amount of enzyme that will generate 1.0 µmol of NADH per min. at pH 8.0 at 37°C.



Figures. a). NADH Standard Curve. **b).** GAPDH activity in HeLa and Jurkat cell lysates. **c).** Specific GAPDH activity were calculated in cell lysates prepared from Jurkat (0.8 µg), HeLa (0.4 µg) and HEK293 cell lysates (0.4 µg). Assays were performed following the kit protocol.

VIII. Related Products:

Fumarate Colorimetric Assay Kit (K633)
Pyruvate Colorimetric /Fluorometric Assay Kit (K609)
Pyruvate Dehydrogenase Activity Assay Kit (K679)
Succinate (Succinic Acid) Colorimetric Assay Kit (K649)
Succinate Dehydrogenase Colorimetric Assay Kit (K660)
Alpha-Ketoglutarate Colorimetric Assay Kit (K677)
Malate Colorimetric Assay Kit (K637)
Malate Dehydrogenase Activity Assay Kit (K645)
Succinyl CoA Synthetase Assay kit (K597)
Isocitrate Colorimetric Assay Kit (K656)
Isocitrate Dehydrogenase Activity Assay Kit (K756)
Aconitase Activity Colorimetric Assay Kit (K716)

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