
Product Manual

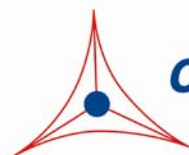
Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) Activity Assay Kit

Catalog Number

MET-5078

200 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures



CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) is a key enzyme of glycolysis and many other cellular processes. Monomeric GAPDH is expressed as a ~37 kDa enzyme, but typically found as a tetramer during most cellular functions (e.g. apoptosis, transcription, membrane trafficking, glucose metabolism). Because of its constitutive expression and ubiquitous cellular/tissue distribution, the GAPDH gene is considered a housekeeping gene. Recently GAPDH has been linked to several neurodegenerative diseases and cancers, making it a potential therapeutic target.

Cell Biolabs' Glyceraldehyde-3-Phosphate Dehydrogenase Activity Kit measures GAPDH activity in cell and tissue samples. GAPDH-containing samples oxidize the kit's substrate in a 2-step, redox reaction; the resulting coenzyme product then reacts with the kit's Colorimetric Probe (absorbance maxima of 450 nm).

The GAPDH Activity Assay Kit is a simple, colorimetric assay that quantitatively measures the GAPDH activity in cell/tissue lysates in a 96-well microtiter plate format. Each kit provides sufficient reagents to perform up to 200 assays, including blanks, standards, and unknown samples. The kit contains an enzyme standard and has a detection sensitivity limit of ~15 mUnits/mL.

Related Products

1. MET-5019: Total Phosphatidic Acid Assay Kit (Fluorometric)
2. MET-5024: Phosphatidylglycerol/Cardiolipin Assay Kit (Fluorometric)
3. MET-5028: DAG (Diacylglycerol) Assay Kit (Fluorometric)
4. MET-5036: DAG Kinase Activity Assay Kit
5. MET-5081: Glucose-6-Phosphate Dehydrogenase (G6PDH) Activity Assay Kit
6. STA-369: OxiSelect™ Human Oxidized LDL ELISA Kit (MDA-LDL Quantitation)
7. STA-390: Total Cholesterol Assay Kit
8. STA-391: HDL and LDL/VLDL Cholesterol Assay Kit
9. STA-394: HDL Cholesterol Assay Kit
10. STA-396: Serum Triglyceride Quantification Kit (Colorimetric)
11. STA-398: Free Glycerol Assay Kit (Colorimetric)
12. STA-618: Free Fatty Acid Assay Kit (Colorimetric)

Kit Components

1. GAPDH Enzyme Standard (Part No. 50781D): One 1 mL vial containing 10 Units/mL of GAPDH.
Note: One unit corresponds to the amount of enzyme which will reduce 1 μ mole of 3-phosphoglycerate to D-glyceraldehyde 3-phosphate per minute at pH 7.6 and 25°C.
2. 5X Lysis Buffer (Part No. 50782B): One 30 mL bottle.
3. Assay Buffer (Part No. 50783B): Three 1.5 mL vials.
4. 5X GAPDH Substrate (Part No. 50784D): Four 1 mL vials.
5. Colorimetric Probe (Part No. 50181C): Two 1 mL amber vials.

Materials Not Supplied

1. PBS
2. 96-well microtiter plate
3. 10 μ L to 1000 μ L adjustable single channel micropipettes with disposable tips
4. 50 μ L to 300 μ L adjustable multichannel micropipette with disposable tips
5. Multichannel micropipette reservoir
6. Microplate reader capable of reading at 450 nm

Storage

Store the entire kit at -80°C. Avoid multiple freeze/thaws by aliquoting. The Colorimetric Probe is light sensitive and should be maintained in amber tubes.

Preparation of Reagents

- 1X Lysis Buffer: Dilute the 5X Lysis Buffer Concentrate to 1X with deionized water. Stir to homogeneity.
- Assay Buffer and Colorimetric Probe: Thaw and maintain at room temperature during assay preparation. Mix well. Any unused material should be aliquoted and frozen at -80°C to avoid multiple freeze/thaws.
- GAPDH Enzyme Standard and 5X GAPDH Substrate: Thaw and maintain at 4°C during assay preparation. Mix well. Any unused material should be aliquoted and frozen at -80°C to avoid multiple freeze/thaws.

Note: 5X GAPDH Substrate is provided in multiple tubes to minimize multiple freeze/thaws.

Preparation of GAPDH Enzyme Standard Curve

- GAPDH Enzyme Standard should be thawed/maintained at 4°C during assay preparation. For longer term storage, the GAPDH Enzyme Standard should be aliquoted and frozen at -80°C to avoid multiple freeze/thaws.
- Freshly prepare a dilution series of GAPDH Enzyme Standard in the concentration range of 1000 mUnits/mL – 15.63 mUnits/mL by diluting the enzyme stock solution (provided at 10 Units/mL) in 1X Lysis Buffer (see Table 1). Standards should be prepared fresh, vortexed well and used immediately. Do not store diluted standards.

Standard Tubes	10 Units/mL GAPDH Enzyme Standard (μL)	1X Lysis Buffer (μL)	Final GAPDH Enzyme Standard (mUnits/mL)
1	50	450	1000
2	250 of Tube #1	250	500
3	250 of Tube #2	250	250
4	250 of Tube #3	250	125
5	250 of Tube #4	250	62.5
6	250 of Tube #5	250	31.25
7	250 of Tube #6	250	15.63
8	0	250	0

Table 1. Preparation of GAPDH Enzyme Standard Curve

Preparation of Samples

- Plasma and serum: This kit is not recommended for these samples.
- Tissue Samples: Weigh out 100 mg of tissue and mince into small pieces. Rinse the tissue with cold PBS to remove red blood cells and clots. Homogenize the minced tissue in 1 mL cold 1X Lysis Buffer. Centrifuge at 10,000 x g for 10 minutes at 4°C. Carefully collect the supernatant and store on ice for immediate use. For longer term storage, freeze the homogenate at -80°C for up to 1 month. Tissue homogenates may need to be further diluted in cold 1X Lysis Buffer before assaying.
- Suspension Cells: Collect 1×10^7 cells by centrifugation at 1000 x g for 10 minutes. Carefully aspirate the culture media and wash once with cold PBS. Centrifuge at 1000 x g for 10 minutes at 4°C. Discard the supernatant and resuspend in 1 mL cold 1X Lysis Buffer. Incubate on ice for 10 minutes. Centrifuge at 10,000 x g for 10 minutes at 4°C. Carefully collect the supernatant and store on ice for immediate use. For longer term storage, freeze the lysate at -80°C for up to 1 month. Cell lysates may need to be further diluted in cold 1X Lysis Buffer before assaying.
- Adherent Cells: Carefully aspirate the culture media and wash once with the recommended volume of PBS (Table 2). Discard the supernatant and add the appropriate volume of cold 1X Lysis Buffer. Incubate on ice for 10 minutes. Transfer the lysate to a microcentrifuge tube. Centrifuge at 10,000 x g for 10 minutes at 4°C. Carefully collect the supernatant and store on ice for immediate use. For longer term storage, freeze the lysate at -80°C for up to 1 month. Cell lysates may need to be further diluted in cold 1X Lysis Buffer before assaying.

Culture Dish	96-well	48-well	24-well
PBS Wash Volume ($\mu\text{L}/\text{well}$)	200	400	800
1X Lysis Buffer ($\mu\text{L}/\text{well}$)	75	150	300

Table 2: Dispensing Volumes of Different Plate Formats

Assay Protocol

Important Note: *Freshly prepare GAPDH Enzyme standards each time the assay is performed.*

Maintain the GAPDH Enzyme Standard and 5X GAPDH Substrate at 4°C during assay preparation.

1. Prepare and mix all reagents thoroughly before use. Each sample, including unknowns and standards, should be assayed in duplicate or triplicate.

Note: Each unknown sample replicate requires two paired wells, one to be treated with 5X GAPDH Substrate (+Sub) and one without (-Sub) to determine background.

2. Add 50 μL of the GAPDH Enzyme standards, samples or blanks to the 96-well microtiter plate.
3. Prepare the desired volume of Substrate Mixture (+Sub) according to Table 3 below, based on the number of tests to be performed. Maintaining all solutions at room temperature, add components **in the following sequence**:
 - a. In a tube, add the appropriate volume of Assay Buffer.
 - b. Next, add the corresponding volume of 5X GAPDH Substrate.
 - c. Finally, add the corresponding volume of Colorimetric Probe. Mix well and immediately use.

Assay Buffer (mL)	5X GAPDH Substrate (mL)	Colorimetric Probe (mL)	Total Volume of Substrate Mixture (mL)	# of Tests in 96-well Plate (50 $\mu\text{L}/\text{well}$)
1	1	0.5	2.5	50
0.5	0.5	0.25	1.25	25
0.2	0.2	0.1	0.5	10

Table 3. Preparation of Substrate Mixture (+Sub)

4. Prepare the desired volume of Control Mixture (-Sub) according to Table 4 below, based on the number of tests to be performed. Maintaining all solutions at room temperature, add components **in the following sequence**:
 - d. In a tube, add the appropriate volume of Assay Buffer.
 - e. Next, add the corresponding volume of deionized water.

- f. Finally, add the corresponding volume of Colorimetric Probe. Mix well and immediately use.

Assay Buffer (mL)	Deionized H ₂ O (mL)	Colorimetric Probe (mL)	Total Volume of Substrate Mixture (mL)	# of Tests in 96-well Plate (50 μ L/well)
1	1	0.5	2.5	50
0.5	0.5	0.25	1.25	25
0.2	0.2	0.1	0.5	10

Table 4. Preparation of Control Mixture (-Sub)

5. Transfer 50 μ L of the Substrate Mixture (+Sub) from step 3 above to all wells containing standards and to one half of the paired sample wells. Mix thoroughly.
6. Transfer 50 μ L of the Control Mixture (-Sub) from step 4 above to the remaining half of the paired sample wells. Mix thoroughly.
7. Incubate at 37°C for 15 minutes.
8. Read the absorbance of each microwell on a spectrophotometer using 450 nm as the primary wavelength.
9. Subtract the OD of the control wells (-Sub) from the OD of the substrate wells (+Sub) to obtain the Net OD for each sample replicate, before comparing to the standard curve.

Example of Results

The following figures demonstrate typical GAPDH Activity Assay Kit results. One should use the data below for reference only. This data should not be used to interpret actual results.

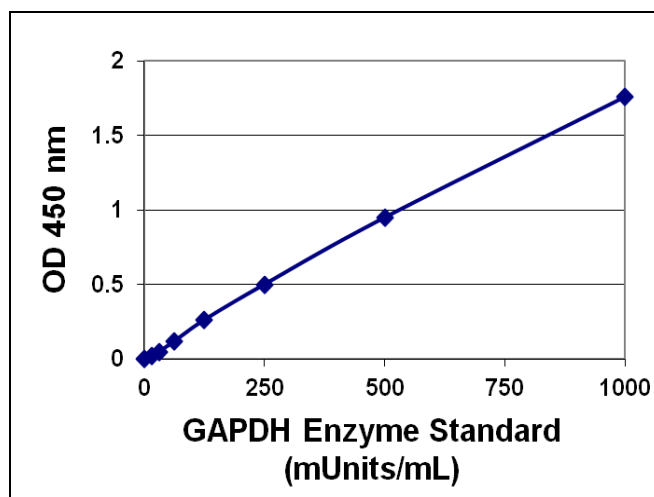


Figure 1: GAPDH Enzyme Standard Curve. GAPDH Enzyme standard curve was performed according to the Assay Protocol. Background has been subtracted.

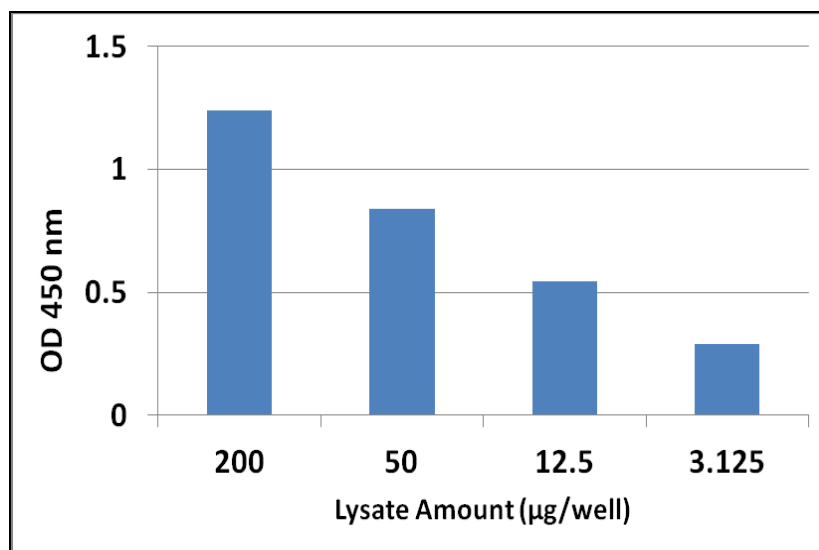


Figure 2: GAPDH Activity of HEK-293 Lysate. HEK-293 lysate was prepared according to the Assay Protocol (total protein concentration was also determined). Background has been subtracted.

References

1. Huang, Y., Zhang, P., Yang, Z., Wang, P., Li, H., and Gao, Z. (2017) *Arch. Biochem. Biophys.* **619**, 54-61.
2. Kosova, A., Khodyreva, S., and Lavrik, O. (2017) *Biochem. (Moscow)* **82**, 643-654.
3. White, M. and Garcin, E. (2017) *Subcell. Biochem.* **83**, 413-453.

Warranty

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Contact Information

Cell Biolabs, Inc.
 7758 Arjons Drive
 San Diego, CA 92126
 Worldwide: +1 858-271-6500
 USA Toll-Free: 1-888-CBL-0505
 E-mail: tech@cellbiolabs.com
www.cellbiolabs.com

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