Product Manual

Glycerol- 3- Phosphate (G3P) Assay Kit (Colorimetric)

Catalog Number

MET- 5075 **100 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

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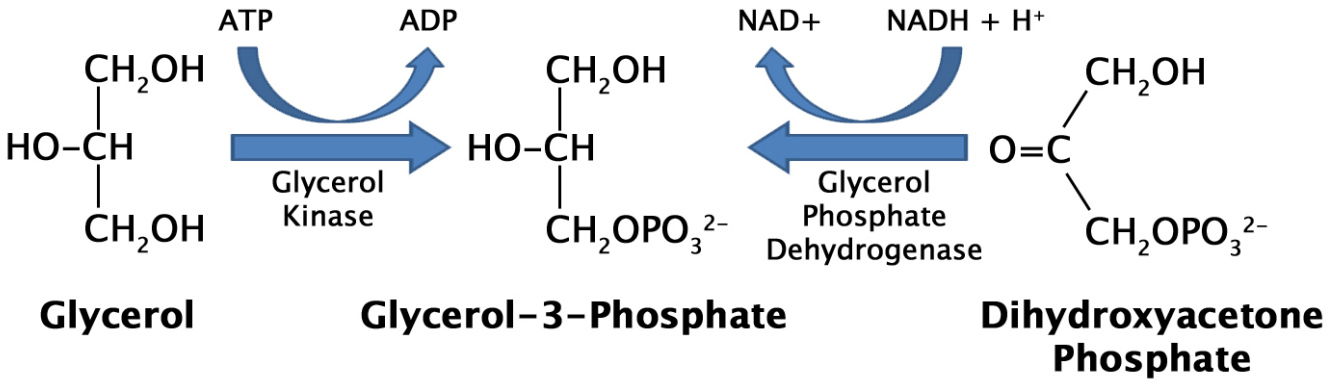
Introduction

Glycerol-3-phosphate (G3P) is a critical precursor to glycerophospholipids, which serve as structural components of cell membranes. Additionally, G3P is an important component of carbohydrate and lipid metabolic processes; it also acts as an electron mediator across the mitochondrial membrane (G3P Shuttle). Glycerol-3-phosphate is produced during glycerol phosphorylation via glycerol kinase. Alternatively, G3P is also generated when glycerol-3-phosphate dehydrogenase (GPDH) reduces hydroxyacetone phosphate (see below).



Cell Biolabs' Glycerol-3-Phosphate Assay Kit measures G3P by an enzymatic, oxidation reaction, producing hydrogen peroxide which reacts with the kit's Colorimetric Probe (absorbance maxima of 570 nm).

The Glycerol-3-Phosphate Assay Kit is a simple, colorimetric assay that quantitatively measures the G3P concentration in various samples using a 96-well microtiter plate format. Each kit provides sufficient reagents to perform up to 100 assays, including blanks, standards and unknown samples. The kit contains a Glycerol-3-Phosphate Standard and has a detection sensitivity limit of ~8 µM (80 pmol/well).

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. STA-369: OxiSelect™ Human Oxidized LDL ELISA Kit (MDA-LDL Quantitation)
6. STA-390: Total Cholesterol Assay Kit
7. STA-397: Serum Triglyceride Quantification Kit (Fluorometric)
8. STA-600: Phosphatidylcholine Assay Kit
9. STA-601: Sphingomyelin Assay Kit
10. STA-619: Free Fatty Acid Assay Kit (Fluorometric)

Kit Components

1. Glycerol-3-Phosphate Standard (50 mM) (Part No. 50751C): One 50 µL vial.
2. 10X Assay Buffer (Part No. 50192D): One 1.5 mL vial.
3. 5X Enzyme Mixture (Part No. 50752D): Four 525 µL vials.
4. 200X Colorimetric Probe (Part No. 239804): One 55 µL amber vial.

Materials Not Supplied

1. 96-well microtiter plate
2. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
3. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
4. Multichannel micropipette reservoir
5. Microplate reader capable of reading in the 540-570 nm range

Storage

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

Preparation of Reagents

- Glycerol-3-Phosphate Standard, 10X Assay Buffer, and 5X Enzyme Mixture should be thawed/maintained at 4ºC during assay preparation. All are stable for 1 week at 4ºC. For longer term storage, each should be aliquoted and frozen at -80ºC to avoid multiple freeze/thaws.

  Note: 5X Enzyme Mixture is provided in multiple tubes to minimize multiple freeze/thaws.

- 200X Colorimetric Probe should be thawed/maintained at room temperature during assay preparation. Any unused material should be aliquoted and frozen at -80ºC to avoid multiple freeze/thaws.

Preparation of Glycerol-3-Phosphate Standard

- Prepare a dilution series of Glycerol-3-Phosphate Standard in the concentration range of 500 µM – 7.81 µM by diluting the stock solution in Deionized Water (Table 1).

Standard Tubes	50 mM Glycerol-3-Phosphate Standard (µL)	Deionized Water (µL)	Final Glycerol-3-Phosphate Standard (µM)
1	5	495	500
2	250 of Tube #1	250	250
3	250 of Tube #2	250	125
4	250 of Tube #3	250	62.5
5	250 of Tube #4	250	31.25
6	250 of Tube #5	250	15.63
7	250 of Tube #6	250	7.81
8	0	250	0

Table 1. Preparation of Glycerol-3-Phosphate Standards

Preparation of Samples

- Cell Lysates: Collect 10 x 10^6 cells by centrifugation at 1000 x g for 10 minutes. Discard the supernatant and resuspend in 1 mL of cold PBS containing 1% Triton X-100. Homogenize or sonicate the cell suspension. Centrifuge at 10000 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.
- Tissue Samples: Weigh out 200 mg of tissue and mince into small pieces. Homogenize the minced tissue in 1 mL of cold PBS containing 1% Triton X-100. Centrifuge at 1000 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.

Assay Protocol

Each G3P standard and sample should be assayed in duplicate or triplicate. A freshly prepared standard curve should be used each time the assay is performed.

1. Add 10 µL of the diluted G3P standards or samples to the 96-well microtiter plate.
2. **Maintain all components/mixtures at 4ºC.** According to Table 2 (below), prepare the desired volume of Reaction Mixture (based on the # of tests) in the following sequence:
    a. In a tube, add the appropriate volume of deionized water.
    b. To the water add the corresponding volume of 10X Assay Buffer. Mix well.
    c. Add the corresponding volume of 5X Enzyme Mixture.
    d. Finally, add the corresponding volume of 200X Colorimetric Probe. Mix well and immediately use.

    Note: Reaction Mixture may appear slightly pink in color. This is normal background and should be subtracted from all absorbance values.

Deionized Water (mL)	10X Assay Buffer (mL)	5X Enzyme Mixture (mL)	200X Colorimetric Probe (µL)	Total Volume of Reaction Mixture (mL)	**# of Tests in 96-well Plate (90 µL/test)**
5.950	1	2	50	9	**100**
2.975	0.5	1	25	4.5	**50**
1.190	0.2	0.4	10	1.8	**20**

Table 2. Preparation of Reaction Mixture

3. Transfer 90 µL of the above Reaction Mixture to each well (already containing 10 µL of G3P standard or sample).
4. Cover the plate wells to protect the reaction from light.
5. Incubate at room temperature for 15 minutes on an orbital shaker.
6. Read absorbance in the 540-570 nm range on a microplate reader.
7. Calculate the concentration of G3P within samples by comparing the sample absorbance to the standard curve. Negative controls (without G3P) should be subtracted.

Example of Results

The following figures demonstrate typical Glycerol-3-Phosphate Assay Kit results. One should use the data below for reference only. This data should not be used to interpret actual results.

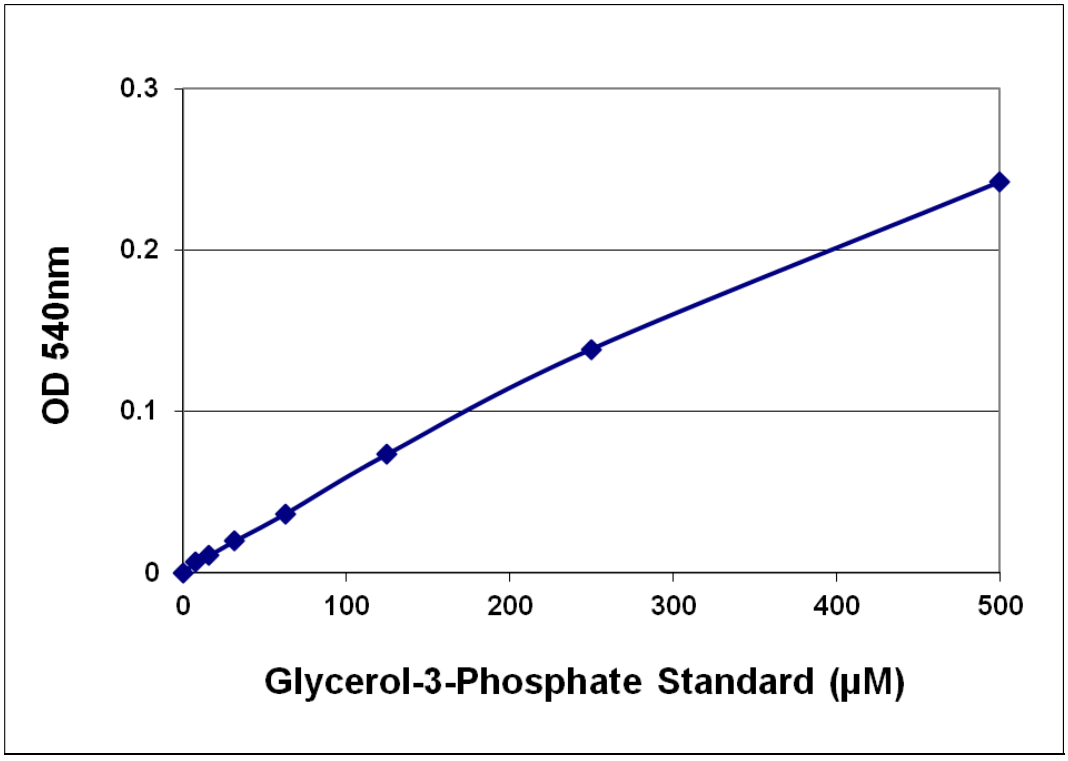


Figure 1: G3P Standard Curve. Glycerol-3-Phosphate standard curve was performed according to the Assay Protocol. Background has been subtracted.

References

1. Vimala, A. and Harinarayanan, R. (2016) *Mol. Microbiol.* **100(2)**, 263-277.
2. Lai, X., Yang, R., Luo, Q., Chen, J., Chen, H., Yan, X. (2015) *J. Phycol.* **51(2)**, 321-331.
3. Larsson, C., Pahlman, I., Ansell, R., Rigoulet, M., Adler, L., and Gustafsson, L. (1998) *Yeast* **14(4)**, 347-357.

Warranty

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