Product Manual

DAG Kinase Activity Assay Kit

Catalog Number

MET- 5036 **50 assays**

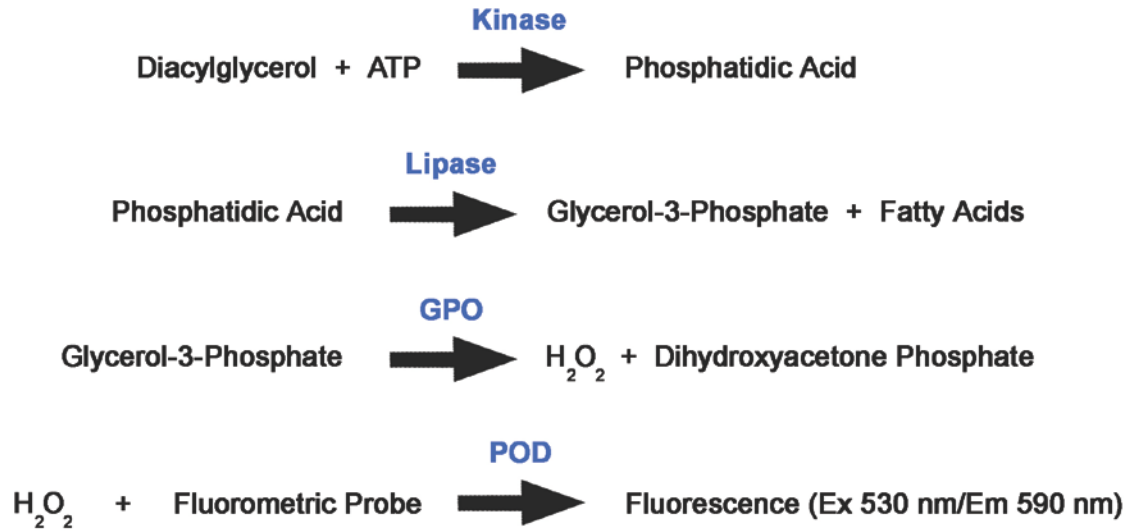
FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Diacylglycerol (DAG) and phosphatidic acid (PA) are key molecules in the biosynthesis of many cellular lipids and play fundamental roles in biochemical signaling. Diacylglycerol acts as a precursor to many lipids (e.g. triglycerides, phospholipids); however, DAG also functions as a second messenger signaling lipid, produced through hydrolysis of PIP2 by phospholipase C, which initiates intracellular Ca^{2+} release and PKC activation. Diacylglycerol kinase (DAGK) is a family of enzymes that phosphorylate diacylglycerol to yield phosphatidic acid, utilizing ATP as a source of phosphate. Therefore, DAGK is thought to be a key regulating enzyme of numerous cellular responses by modulating the balance between these two signaling lipids.

Cell Biolabs' DAG Kinase Activity Assay Kit measures DAGK activity in samples by a coupled enzymatic reaction system. First, kinase-containing sample is used to phosphorylate DAG substrate, yielding phosphatidic acid. Next, a lipase is used to hydrolyze phosphatidic acid to glycerol-3-phosphate. Finally, the glycerol-3-phosphate product is oxidized by glycerol-3-phosphate oxidase (GPO), producing hydrogen peroxide which reacts with the kit's Fluorometric Probe (Ex. 530-560 nm/Em. 585-595 nm).



The DAG Kinase Activity Assay Kit is a non-isotopic, fluorometric assay that quantitatively measures DAGK activity using its physiological substrate (diacylglycerol) in a 96-well microtiter plate format. Recombinant DAGK is also provided as a positive control. Each kit provides sufficient reagents to perform up to 50 assays, including blanks, standards and unknown samples.

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-5035: Recombinant Diacylglycerol Kinase (*E. coli*)

5. STA-369: OxiSelect™ Human Oxidized LDL ELISA Kit (MDA-LDL Quantitation)
6. STA-390: Total Cholesterol Assay Kit
7. STA-391: HDL and LDL/VLDL Cholesterol Assay Kit
8. STA-394: HDL Cholesterol Assay Kit
9. STA-397: Serum Triglyceride Quantification Kit (Fluorometric)
10. STA-399: Free Glycerol Assay Kit (Fluorometric)
11. STA-600: Phosphatidylcholine Assay Kit
12. STA-601: Sphingomyelin Assay Kit
13. STA-619: Free Fatty Acid Assay Kit (Fluorometric)

Kit Components

1. DAG Substrate (Part No. 50361D): One 1 mL vial containing 10 mM 1-2-dioleoyl-s*n*-glycerol.
2. 4X Assay Buffer (Part No. 50362D): Two 1.5 mL vials containing octyl β-D-glucopyranoside and DETAPAC.
3. Kinase Buffer (Part No. 50363D): One 500 µL vial containing ATP, octyl β-D-glucopyranoside, and DETAPAC.
4. Lipase Solution (Part No. 50193D): Two 1.4 mL vials.
5. Enzyme Mixture (Part No. 50194D): Two 1.75 mL vials.
6. 200X Fluorometric Probe (Part No. 239901): One 55 µL amber vial.
7. Phosphatidic Acid Standard (Part No. 50364D): One 200 µL vial of 1 mM L-α-Phosphatidic Acid.
8. Recombinant DAGK (Part No. 50365D): One 100 µL vial containing 10 µg of Recombinant DAGK from *E. coli*.

Materials Not Supplied

1. Standard 96-well fluorescence black microtiter plate
2. PBS (containing Magnesium and Calcium)
3. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
4. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
5. Multichannel micropipette reservoir
6. Fluorescence microplate reader capable of reading excitation in the 530-560 nm range and emission in the 585-595 nm range

Storage

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

Preparation of Reagents

- DAG Substrate and Phosphatidic Acid Standard: Thaw at 37ºC for 30 minutes. Mix well and maintain the solution at 37ºC during assay preparation. Any unused material should be aliquoted and frozen at -80ºC to avoid multiple freeze/thaws. Repeat 37ºC thawing procedure for any aliquoted material.
- 1X Assay Buffer: 4X Assay Buffer should be thawed/maintained at 4ºC during assay preparation. Dilute the 4X Assay Buffer with deionized water. Stir to homogeneity. The 1X solution is stable for 1 month at 4ºC. For longer term storage, any unused 4X stock material should be aliquoted and frozen at -80ºC to avoid multiple freeze/thaws.
- Kinase Buffer and Recombinant DAGK: Thaw and maintain at 4ºC during assay preparation. Any unused material should be aliquoted and frozen at -80ºC to avoid multiple freeze/thaws.
- Lipase Solution and Enzyme Mixture: Thaw at 4ºC. Once homogeneous and mixed well, maintain the solution at 4ºC during assay preparation. The solution is stable for 1 week at 4ºC. For longer term storage, the solution should be aliquoted and frozen at -80ºC to avoid multiple freeze/thaws.

  Note: These components are provided in multiple tubes to minimize multiple freeze/thaws.
- 200X Fluorometric Probe: Thaw and maintain at room temperature during assay preparation. Any unused material should be aliquoted and frozen at -80ºC to avoid multiple freeze/thaws.

Preparation of Phosphatidic Acid Standard (optional)

Thaw the Phosphatidic Acid Standard at 37ºC (see Preparation of Reagents). Mix well by vortexing to ensure the solution is homogeneous. Freshly prepare a dilution series of standard in the concentration range of 0 µM – 1 mM by diluting the standard stock solution (provided at 1 mM) in 1X Assay Buffer (see Table 1). Standards should be prepared fresh, vortexed well and used immediately.

Standard Tubes	1 mM Phosphatidic Acid Standard (µL)	1X Assay Buffer (µL)	Final Phosphatidic Acid Standard (µM)
1	75	0	1000
2	75 of Tube #1	75	500
3	75 of Tube #2	75	250
4	75 of Tube #3	75	125
5	75 of Tube #4	75	62.5
6	75 of Tube #5	75	31.25
7	75 of Tube #6	75	15.63
8	0	75	0

Table 1. Preparation of Phosphatidic Acid Standards

Assay Protocol

Important Note: Maintain the kit's Recombinant DAGK, Kinase Buffer, Lipase Solution, and Enzyme Mixture at 4ºC during assay preparation. Each DAGK sample should be assayed in duplicate or triplicate.

1. If running the optional Phosphatidic Acid Standard Curve (see Figure 2), add 20 µL of each standard from Table 1 to wells of a 96-well plate. Add 10 µL of Kinase Buffer and 10 µL of PBS to each well and mix thoroughly.
2. Separately, add 20 µL of DAG Substrate for each test and control well. Add 10 µL of Kinase Buffer. Then add 10 µL of DAGK unknown sample, blank, or Recombinant DAGK (Positive Control) and mix thoroughly.
3. Incubate at 37ºC for 30 minutes.
4. Transfer 20 µL of the mixture to a 96-well plate suitable for fluorescence measurement.
5. Add 40 µL of Lipase Solution to each well.
6. Incubate at 37ºC for 30 minutes.
7. During the step 6 incubation, separately prepare the desired volume of Detection Enzyme Mixture according to Table 2 below, based on the number of tests to be performed. Maintaining all components and mixtures at 4ºC throughout this step, add components in the following sequence:
   a. In a tube, add the appropriate volume of Enzyme Mixture.
   b. To the Enzyme Mixture, add the corresponding volume of 200X Fluorometric Probe. Mix well and immediately use.

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

Enzyme Mixture (mL)	200X Fluorometric Probe (µL)	Total Volume of Detection Enzyme Mixture (mL)	**# of Tests in 96-well Plate (100 µL/test)**
2.5	25	2.525	**50**
1.25	13	1.263	**25**
0.5	5	0.505	**10**

Table 2. Preparation of Detection Enzyme Mixture

8. Transfer 50 µL of the above Detection Enzyme Mixture (from step 8) to each well.
9. Cover the plate wells to protect the reaction from light.
10. Incubate at room temperature for 10 minutes.
11. Read the plate with a fluorescence microplate reader equipped for excitation in the 530-560 nm range and for emission in the 585-595 nm range.

Example of Results

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

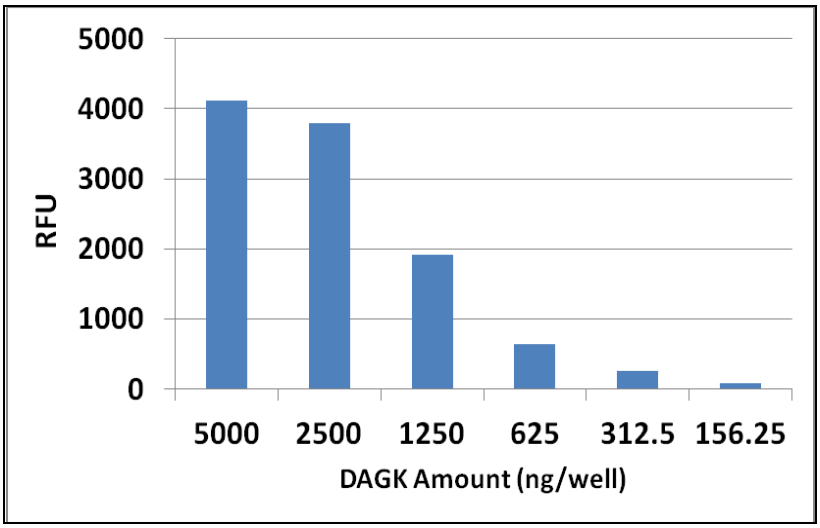


Figure 1: DAGK Activity Assay. Various amounts of rDAGK were incubated for 30 minutes at 37ºC, according to the Assay Protocol. Negative control values (without DAGK) have been subtracted.

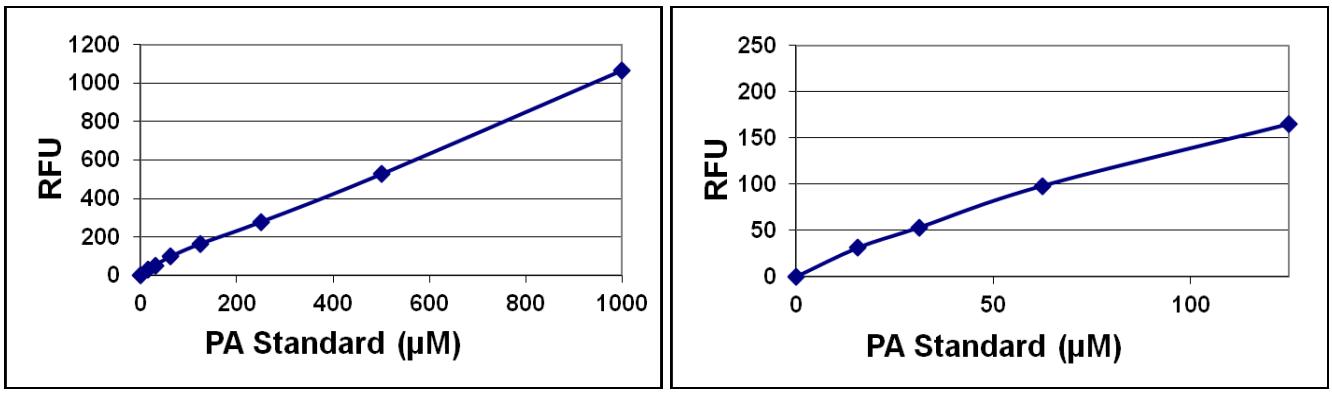


Figure 2: PA Standard Curve. PA standard curve was performed according to the Assay Protocol. Background has been subtracted.

References

1. Yang, C. and Kazanietz, M.G. (2003) *Trends Pharmacol. Sci.* **24**, 602-608.
2. Brose, N. et al. (2004) *Curr. Opin. Neurobiol.* **14**, 328-340.
3. Athenstaedt, K. and Daum, G. (1999) *Eur. J. Biochem.* **266**, 1-16.
4. Kanoh, H. et al. (2002). *J. Biochem. (Tokyo)* **131**, 629-633.
5. Corbalan-Garcia, S. and Gomez-Fernandez, J.C. (2006) *Biochim. Biophys. Acta* **1761**, 633-654
6. Wang, Q.J. (2006). *Trends Pharmacol. Sci.* **27**, 317-323.

Warranty

These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

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