B-Bridge International, Inc.

GPDH Activity Assay

Measure glycerol-3-phosphate dehydrogenase in precursor adipocytes

Catalog # PMC-AK01-COS

For research use only
Not for diagnostic use

V121213

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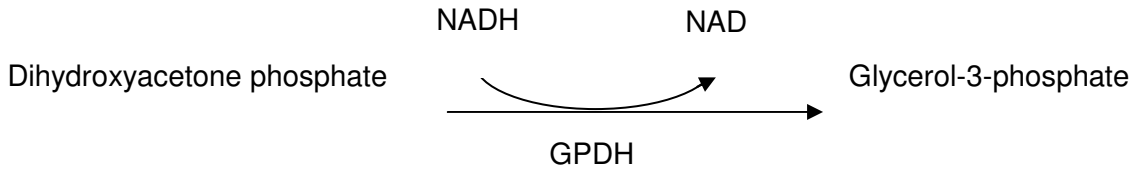
Purpose

GPDH Activity Assay is a quantitative colorimetric measurement of glycerol-3-phosphate dehydrogenase (GPDH) activity in cell cultures and tissue samples such as adipocytes and adipose tissues.

Introduction

An organism's major sources of fatty acids come from its diet or mobilization from cellular storage. Fatty acids from the diet are solubilized and absorbed through the gut and delivered to cells via blood transport. Excess free state long chain fatty acids are cytotoxic in cells. Adipocytes avoid the accumulation of fatty acids by storing it in the form of triacylglycerols. In adipose tissue, GPDH reduces dihydroxyactone phosphate (DHAP) to glycerol 3-phosphate using coenzyme NAD. The sequential binding of three glycerol 3-phosphates by coenzyme acyl-CoA generates triacylglycerol. In response to energy demands, the fatty acids stored as triacylglycerols can be utilized by peripheral tissues.



The measurement of GPDH activity is often used as a marker for lipogenesis (biosynthesis of fat). The activity of GPDH rapidly increases upon differentiation of precursor adipocytes to mature adipocytes.

The GPDH Activity Assay is a rapid and easy to use assay to quantify GPDH in cultured cells or tissue samples. When exogenous DHAP and NADH are mixed with the test sample, glycerol 3-phosphate and NAD will be produced, if the sample contains GPDH activity. GPDH activity is measured by the decrease concentration of NADH.

Components

Kit components can be stored at -20℃ prior to use

- Substrate Solution containing DHAP and NADH, lyophilized……..10 bottles
- Enzyme Extraction Reagent, powder…….1 bag

1 kit = 100 tests

Additional materials and equipment may be required

- Distilled water
- Pipette
- PBS
- Spectrophotometer with 340 nm wavelength
- Quartz microcuvette
- Centrifuge
- Centrifuge tubes
- Sonicator

Protocol

I. Reagent preparation and storage

Prepare and store reagents at 4℃

1. Reconstitute the lyophilized Substrate Solution in 4.2 ml purified water per bottle. The solution is stable for 2 days at 4℃. Only reconstitute the number of bottles that will be used immediately.
2. Dissolve the Enzyme Extraction Reagent in 200 ml distilled water. The solution is stable for 4 weeks at 4℃.

Note: Do not mix reagents from different kits unless they have the same lot number.

II. Sample Preparation

Samples should be prepared and maintained at 4℃

1. Tissue sample
    i. Homogenize 1 g of adipose tissue in 4 ml of 0.25M sucrose
    ii. Centrifuged at 700 x g for 10 minutes at 4℃
    iii. If a fat layer forms on the surface of the sample, carefully remove the fat layer.
    iv. Transfer the supernatant (cytosol fraction) to a tube and dilute 20 to 100 times with the Enzyme Extraction Reagent, then assay samples.

2. Culture cells
    i. Remove culture medium and wash the cells 2 times with 500ul PBS.
    ii. Add Enzyme Extraction Reagent to each well. For a 24-well plate, use 0.5 – 1 ml per well.
    iii. Scrape cells with a sterile rubber policeman. Transfer cells to a clean centrifuge tube

iv. Using a sonicator, homogenize the cell extracts
v. Crude extracts may be directly assayed or centrifuged at 12,800 x g for 5 minutes at 4℃. Centrifugation is recommended.
vi. Assay the sample supernatant

III. Assay Procedure

1. Add 400 µl of resuspended Substrate Solution to a quartz microcuvette. Bring the solution to room temperature. If the spectrophotometer has an incubator, incubate 5 minutes at 25℃.
2. Allow samples to equilibrate to room temperature
3. Add 200 µl of sample to the cuvette containing the Substrate Solution. Mix well.
4. Use the kinetic analysis mode of the spectrophotometer or manually measure OD_{340nm} starting at time 0 and every 30 seconds for 3 minutes.

Alternative: 24-well and 96-well plate format

1. Add 800 µl Substrate Solution per well (24-well) or 80 µl per well (96-well). Bring the solution to room temperature.
2. Allow samples to equilibrate to room temperature
3. Add 400 µl of sample per well (24-well) or 40 µl per well (96-well). Gently mix, do not create bubbles.
4. Use the kinetic analysis mode of the spectrophotometer or manually measure OD_{340nm} starting at time 0 and every 30 seconds for 3 minutes.

IV. Calculation of GPDH activity

1. Plot OD_{340nm} against time
2. Using the linear range of the graph, select 2 time points that are 1 minute apart (i.e. time points 60 and 120 seconds) to calculate $\Delta OD_{340nm/min}$

$$\text{GPDH activity (U/ml)} = \frac{\triangle OD_{340nm/min} \times A}{6.22 \times B \times C}$$

A (ml) = Total reaction volume
6.22 = Millimolar absorption coefficient of NADH molecules
B (ml) = Sample volume assayed
C (cm) = Optical path length

If followed dilutions in Section III,
A= 600 ml
B= 200 ml
C = 1 cm

If using a microplate then,

$$C = \frac{\text{Sample volume in the well (ml)}}{\text{Bottom surface area of well (cm}^2\text{)}}$$

3. 1 Unit of GPDH activity is defined as 1 ml of sample consumes 1 µmole of NADH in 1 minute (light path = 1cm).

Application Notes for cultured adipocytes

Primary culture from adipose tissue or cell lines (i.e. 3T3-L1, 3T3-f442, ob1771)

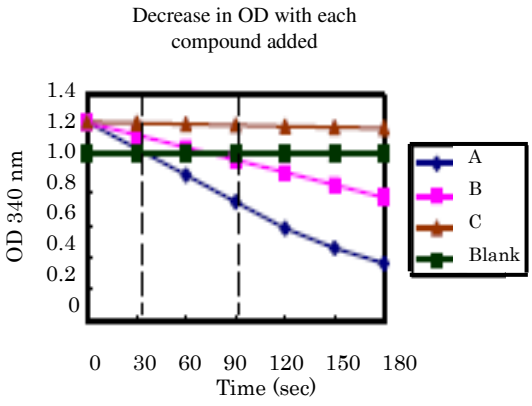
Culture medium:

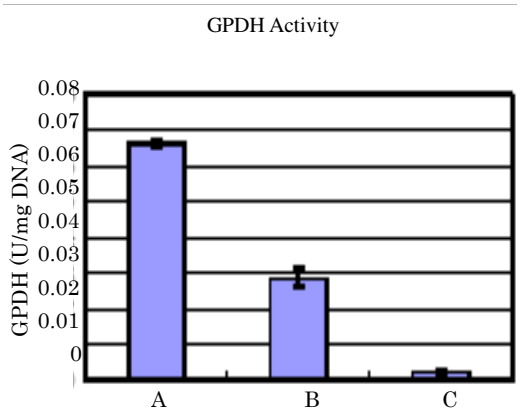
1. Basal Medium: DMEM containing high concentration of glucose (4.50 g/l high glucose) with 10%FBS
2. Differentiation Medium: Basal medium + 0.25 µM Dexamethazone and 10 µg/ml Insulin

Culture protocol:

1. Plate cells at 0.5 – 1 X 10^5 cells/well in a 24-well plate. It takes approximately 1-2 days for cells to become confluent
2. Once culture is confluent, replace the Basal Medium with the Differentiation Medium
3. Incubate for 2 days
4. Replace the medium with Basal Medium
5. Add test compounds, such as inhibitors and inducers for lipid accumulation
6. Incubate 5 - 10 days until lipid accumulates in the cells
7. Wash cells 2 times using PBS
8. Add 0.5 – 1 ml Enzyme Extraction Solution to each well
9. Remove cells with a rubber policeman and place the cells in a tube
10. Sonicate cells on ice
11. Perform GPDH assay on the extract.

Example Data





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