KAMIYA BIOMEDICAL COMPANY

GPDH Activity Measurement Kit

For quantitative colorimetric determination of GPDH (glycerol-3-phosphate dehydrogenase) activity in tissue samples and cultured cells such as adipose tissues and adipocytes.

Cat. No. KT-010

For Research Use Only. Not for Use in Diagnostic Procedures.

PRODUCT INFORMATION

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PRODUCT
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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 the 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 dihydroxyacetone 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.

PRINCIPLE
The measurement of GPDH activity is widely used to assess the biosynthesis of fat in adipocytes and adipose tissues. The activity of GPDH rapidly increases upon differentiation of precursor adipocytes to mature adipocytes. The **K-ASSAY**® GPDH Activity Measurement Kit offers a simple and rapid assay procedure for assaying tissue samples or cultured cells for GPDH. When DHAP (dihydroxyacetone phosphate) and NADH are mixed in the presence of GPDH from the sample, glycerol-3-phoshate and NAD are produced. The decrease in NADH concentration is then measured at 340 nm.

$$\text{DHAP} + \text{NADH} \xrightarrow{\text{GPDH}} \text{Glycerol-3-phosphate} + \text{NAD}$$

COMPONENTS
- 10 x Substrate Reagent, Lyophilized, contains DHAP and NADH
- 1 x Enzyme Extracting Reagent, Lyophilized

1 kit = 100 tests (when using a 96-well microplate, up to 500 tests can be run)

Materials required but not provided
- Purified water
- Adjustable pipettor
- PBS
- Spectrophotometer or microplate reader capable of measuring at 340 nm
- Quartz cuvette for spectrophotometer method or microtiter plate for microplate method
- Centrifuge
- Centrifuge tubes
- Sonicator

PROTOCOLS
Reconstitution of Components
Follow the directions carefully to ensure that the proper volumes of water are used to reconstitute each component.
1. Reconstitute the lyophilized Substrate Solution in 4.2 mL purified water per bottle. The solution is stable for 2 days at 4°C. Only reconstitute the number of lyophilized Substrate Solution bottles that will be used immediately.
2. Dissolve the entire contents of the Enzyme Extracting Reagent pouch in 200 mL of purified water. The solution is stable for 4 weeks at 4°C.

Sample Preparation

1. Tissue Sample:
   a. Add 4 mL of 0.25M sucrose solution to 1 gram of adipose or other tissue and homogenize the mixture.
   b. Centrifuge at 700 x g at 4°C for 10 minutes. Remove the supernatant (do not discard).
   c. Centrifuge the supernatant at 54,000 x g at 4°C for 60 minutes. Remove the supernatant (cytosol fraction).
   d. Dilute the supernatant approximately 20 to 100 times with reconstituted Enzyme Extracting Reagent.
   e. Assay the sample.

2. Cultured Cells:
   a. Remove culture medium and wash sample cells twice with PBS.
   b. Add the reconstituted Enzyme Extracting Reagent to the sample.
   c. When a 24-well plate is used, add the reconstituted Enzyme Extracting Reagent at 0.5 to 1.0 mL per well.
   d. Scrape cells with a sterile rubber policeman. Transfer cells to a clean centrifuge tube.
   e. Use a sonicator to homogenize the cell extracts.
   f. Crude extracts may be directly assayed or centrifuged at 12,800 x g at 4°C for 5 minutes. Centrifugation is recommended.
   g. Use the supernatant as the sample for the assay.

Assay Procedure

1. Dispense 400 µL of the reconstituted Substrate Reagent into a quartz micro-cuvette. Bring the solution to room temperature. If the spectrophotometer has an incubator, incubate for 5 minutes at 25°C.
2. Bring samples to room temperature.
3. Add 200 µL of the sample to the cuvette containing the Substrate Solution and mix well.
4. Use the kinetic analysis mode of the spectrophotometer or manually measure the OD at 340 nm for 3 – 10 minutes to calculate the ΔOD/minute (change in optical density at wavelength 340 nm).

Calculation of GPDH Activity

1. Plot OD against time
2. Use the linear range of the graph to calculate ΔOD
3. 1 Unit of GPDH activity is defined as 1 mL of sample consumes 1 µmole of NADH in 1 minute (light path = 1 cm)
   GPDH activity (U/mL) = ΔOD at 340 nm/minute x 0.482

Application Notes for Cultured Adipocytes:

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

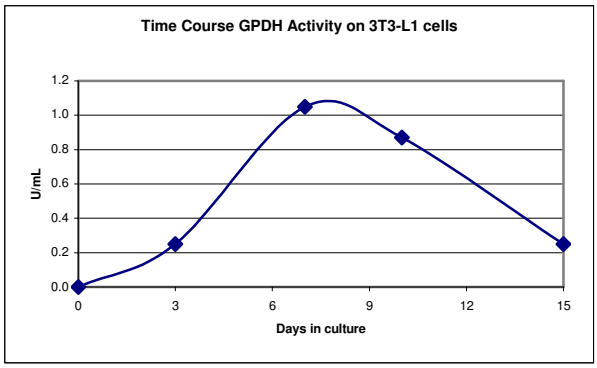
Culture Medium:

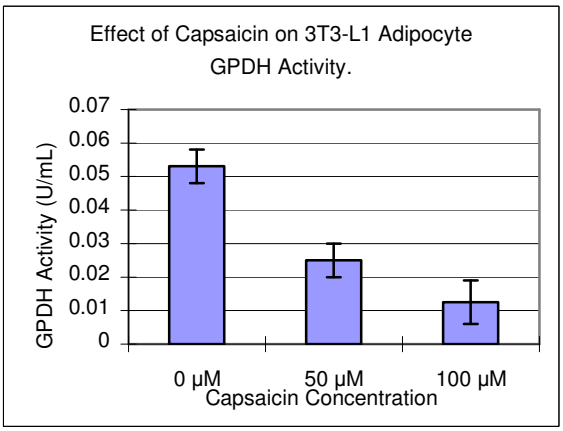
1. Basal culture medium: DMEM containing high concentration of glucose (4.5 g/L high glucose) with 10% FBS.
2. Differentiation medium: Add 0.25 µM dexamethasone and 10 µg/mL insulin to the above basal culture medium.

Culture Protocol:

1. Plate cells at 0.5 - 1 x 10^5 cells per well into a 24-well plate. Wait for the cells to become confluent. Typical time is 1 to 2 days.
2. Once the culture is confluent, replace the Basal Medium with the Differentiation Medium.
3. Incubate for 2 days.
4. Replace the Differentiation Medium with Basal Medium.
5. Add test compounds, such as inhibitors and inducers of lipid accumulation.
6. Incubate 5 – 10 days until lipid accumulates in the cells.
7. Wash cells 2 times with PBS.
8. Add 0.5 - 1.0 mL of reconstituted Enzyme Extracting 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.

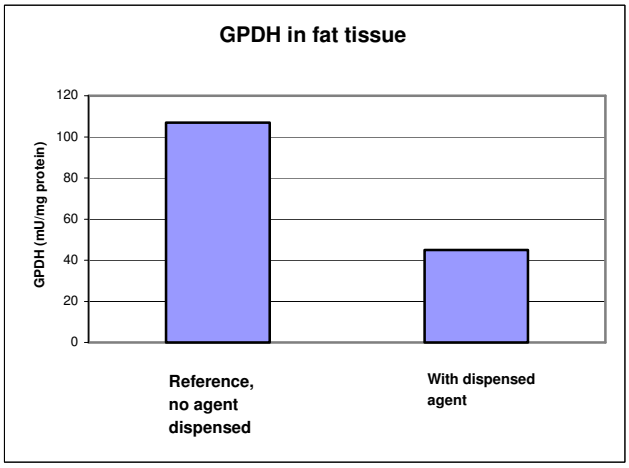
Example Data

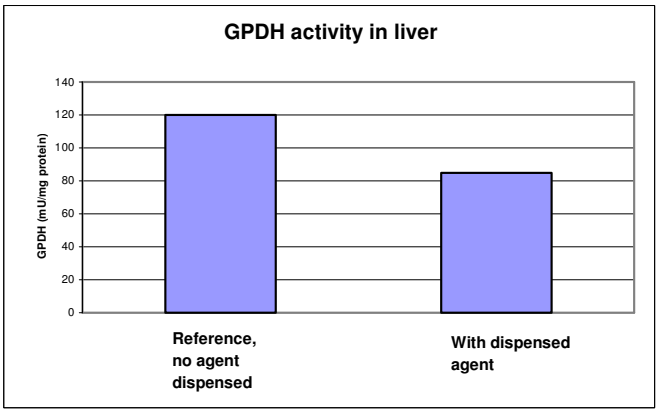




Measurement of GPDH activity in rat tissue

A substance (TBE) which includes a fat deposit accumulation inhibitor was orally dispensed to rats. The GPDH activity in the fat tissue surrounding the kidney and in the liver was measured and compared to a reference sample to which the agent was not dispensed.





STORAGE

Kit components can be stored at -20°C until expiration date. Upon reconstitution, store the Enzyme Extracting Reagent at 4°C for up to 4 weeks and the Substrate Reagent at 4°C for up to 2 days.

PRECAUTIONS

1. Read the instructions carefully before beginning the assay.
2. This kit is for research use only, not for human or diagnostic use.
3. Great care has been taken to ensure the quality and reliability of this product. However, it is possible that in certain cases, unusual results may be obtained due to high levels of interfering factors.

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NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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