Glycerol-3-phosphate Dehydrogenase Assay (GPDH)

Cat. No. 8508
100 Tests in 96-well plate

Introduction

Glycerol-3-phosphate dehydrogenase (GPDH), the enzyme that catalyzes the reversible conversion between dihydroxyacetone phosphate and glycerol 3-phosphate, plays an important role in lipid biosynthesis. The measurement of GPDH activity is useful in assessing adipocyte differentiation and lipid biosynthesis in adipose and muscle tissues. This colorimetric assay is based on the oxidization of NADH to NAD in the presence of dihydroxyacetone phosphate and GPDH. The GPDH activity is determined by assaying the rate of NADH oxidation, which is proportional to the reduction in absorbance at 340nm over time (ΔOD_{340nm}/min).

Kit Components

Cat. No.	# of vials	Reagent	Quantity	Storage
8508a	1	Homogenization buffer	10 mL	4°C
8508b	1	Assay buffer	10mL	4°C
8508c	1	GPDH positive control	10µL	-80°C
8508d	1	Substrate	1.0 mL	-20°C
8508e	1	Cofactor	0.2 mL	-20°C

Product Use

The GPDH Assay kit measures GPDH activity of different types of samples, such as tissues and cell lysate. This product is for research purposes only and not for use in animals, humans, or diagnostic procedures.

Quality Control

Diluted GPDH positive control is measured with the GPDH Assay kit after different time of reaction (Figure 1 and 2). The detection limitation is from 0.00675 to 0.108 U/mL.

Shipping

Shipped on dry ice.

Reagents Supplied by User

1. Beta-mercaptoethanol
2. Ultrapure water (ScienCell™ Cat. No. 0600)

Reagents and Positive Control Preparation

1. Beta-mercaptoethanol: Add 1 µL beta-mercaptoethanol into 143 µL ultrapure water, and subsequently dilute 100-fold in homogenization buffer (8508a) and assay buffer (8508b) before use.
2. Diluted GPDH positive control: Add 1 µl of GPDH positive control into 99 µl assay buffer (8508b).Prepare diluted GPDH positive control to a final volume of 10 µL/well on the 96-well flat bottom plate.

Procedure (96-well plate)

A. Preparation of test samples and blank

1. Cell or tissues can be homogenized in 4 volumes of the homogenization buffer (8508a). Centrifuge the samples at 10,000 ×g for 10 minutes at 4ºC to remove insoluble material. The soluble fraction may be assayed directly.
2. Samples should be serial diluted to make sure the readings are within the standard curve range. Prepare test samples to a final volume of 10 µL/well on the 96-well flat bottom plate.
3. Prepare blank by adding 10 µL assay buffer (8508b) into one well on the 96-well flat bottom plate.

B. Working reagent preparation and measurements

1. Prepare appropriate volume of GPDH assay working reagent based on the number of samples to be measured. For each well of reaction, prepare working reagent by mixing 78µL assay buffer (8508b), 10µLsubstrate (8508d) and 2 µL cofactor (8508e).
2. Add 90 µL of working reagent mix into each well of the 96-well plate containing diluted GPDH positive control, samples and blank, mix well immediately and start recording OD_{340nm} over a 3 minutes interval, collecting data every 0.5 min. Figure 1 shows data of GPDH positive control.

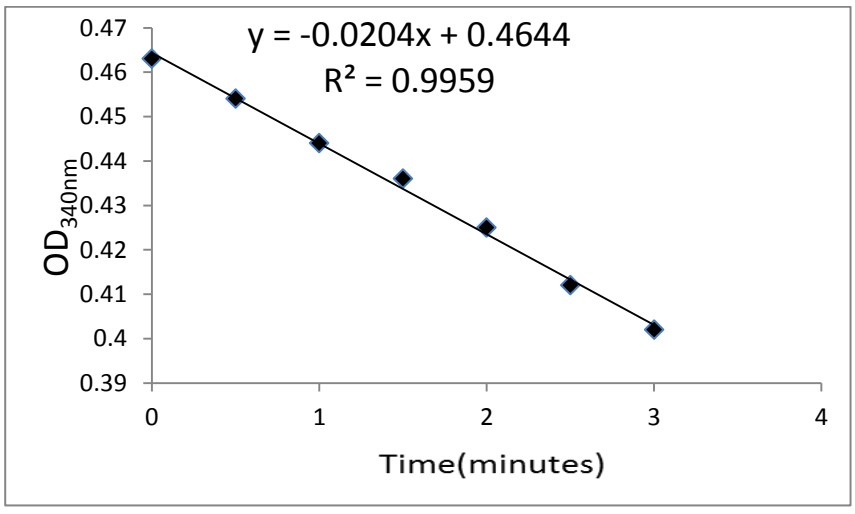

Figure.1 Absorbance change of diluted GPDH positive control at 340nm.

C. Calculations

1. Determine the change in absorbance ΔOD_{340nm}/min by plotting the absorbance value at ΔOD_{340nm} as a function of reaction time to obtain the slope of the linear portion of the curve. As shown in Figure.2.

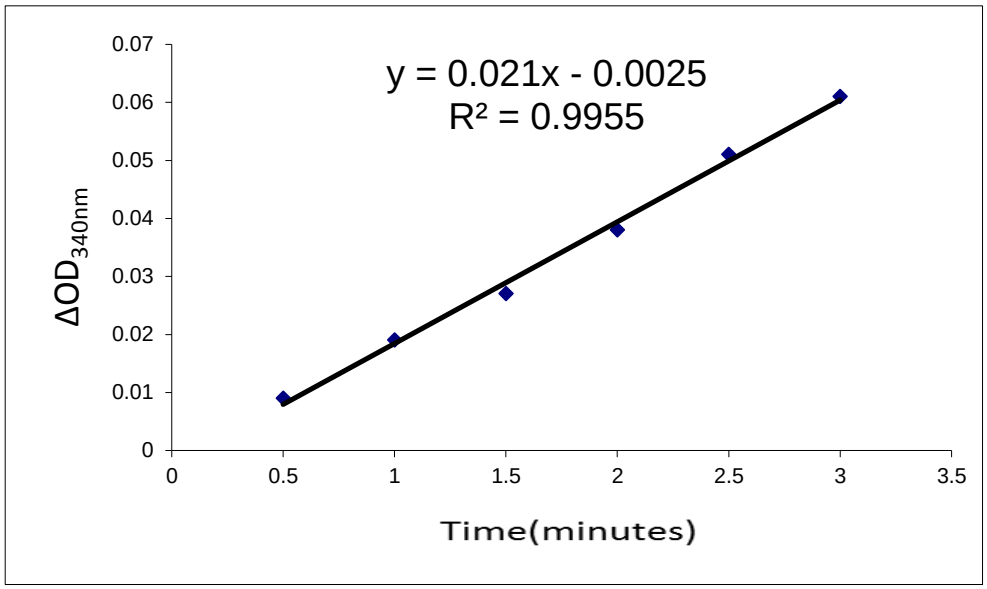

Figure.2 The change in absorbance ΔOD_{340nm} of diluted GPDH positive control during the time at 340nm.

2. Calculate the GPDH activity using the following formula:

$$\text{GPDH (U/ml)} = \frac{\Delta OD_{340}/\text{min} \times 100\ \mu l}{1.94 mM^{-1} \times 10\ \mu l} \times \text{sample dilution}$$

Note: The actual extinction coefficient for NADH at 340nm is $6.22 mM^{-1}cm^{-1}$. This value has been adjusted for the path length of the solution in the 96-well plate.

Unit definition: One unit would make 1.0 µmol of NADH to NAD^+ per minute at pH 7.4 at 25 ºC

3. Use the formula to calculate GPDH positive control activity:

$$\text{GPDH positive control(U/ml)} = \frac{0.021 \times 100\ \mu l}{1.94 mM^{-1} \times 10\ \mu l} \times 100 = 10.82\ \text{(U/ml)}$$