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Inspiration for Life Science

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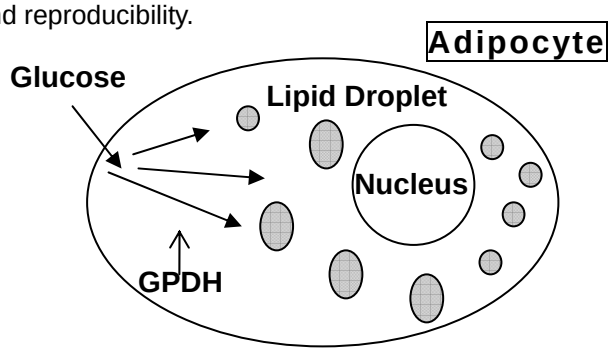
Catalog No. PMC-AK01-COS

GPDH Assay Kit

Introduction

The adipose tissue works as a place energy store in vivo. The way to do that is turning the energy derived from foods into fat by enzymes has a role in fat synthesis. Among the enzymes, glycerol 3 –phosphate dehydrogenase (GPDH) is most measured enzyme that to analyze fat synthesis activity of adipose tissue and adipocyte.
This kit has several advantages, in measuring the enzyme activity, over the traditional methods, which include higher stability and reproducibility.



Principle

Dihydroxyacetone-phosphate + NADH →→→ Glycerol-3-phosphate + NAD
The decrease of NADH is measured at 340nm by spectrophotometry.

Components

Component	Quantity	Form	Stability
SUBSTRATE	10 bottles (1 bottle=10 tests)	Freeze Dry	-20°C
ENZYME EXTRACTION REAGENT	1 bag	Powder	-20°C

One kit contains regents for 100 samples

Regent preparation

・SUBSTRATE SOLUTION
Add one bottle of SUBSTRATE to 4.2 mL of distilled water and mix.
Note: This working solution can be stored at 4°C for 2 days.

・ENZYME EXTRACTION SOLUTION
Add one bag of ENZYME EXTRACTION REAGENT to 200 mL of distilled water and mix.
Note: This working solution can be stored at 4°C for 4 weeks.

Sample preparation - cultured cells

1. Remove culture medium and wash the cells twice with 500µl PBS.
2. Add enzyme extraction solution to each well. For a 24-well plate, apply 0.5~1mL per well.
3. Homogenize the cell extract by using a sonicator.

Assay procedure

1. Add 400µL of substrate solution to spectrometer cuvette (quarts micro cuvette), and warm at 25°C (About 5 minutes). Hopefully use spectrometer keep warm at 25°C. When couldn't, wait until substrate solution become room temperature. In same way, warm samples at 25°C.
2. Add 200µL of sample to cuvette and mix it well. Measure decrease of absorbance at 340nm, and find amount of absorbance change per minute (⊿O.D.). Use kinetic program on spectrometer. If don't have it, time measurement with timer. In general, measure for 3~10minutes.

Note 1: As described in example data, find slope (⊿O.D.) on linearity area.

Note 2: It is possible to measure for a maximum of 500 samples, if use 96well micro-plates reader.

Calculation of GPDH activity

One unit is activity of 1µmol NADH example by GPDH per minute per 1mL sample. Based on this, GPDH activity is found following equation. (Only light path is 1cm)

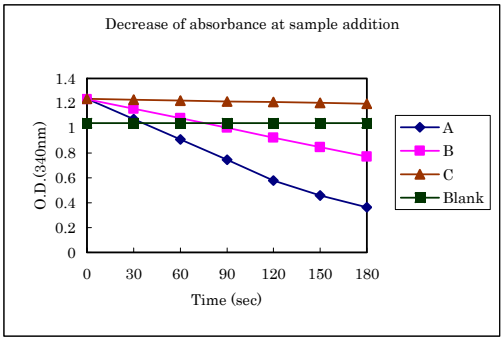
GPDH activity (U/ml) = ⊿O.D.(340nm)/min × 0.482 *

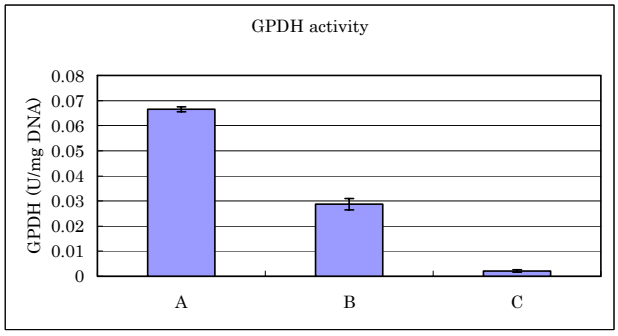
(⊿O.D. : value of absorbance change at 340nm)

*96well plate

Light path (cm) = total amount of reaction (mL) /area of the bottom of well (cm^2)

Example data





References

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(4) Jiao, WH., Gao, H., Li, CY., Zhou, GX., Kitanaka, S., Ohmurae, A., Yao, XS. beta-Carboline Alkaloids from The Stems of Picrasma quassioides. Magn. Reson. Chem. 48, 490-495 (2010)

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