Glycerol-3-phosphate Dehydrogenase (GPDH) Assay Kit FAQs

Cat.# MK426

I do not have a 96-well microplate reader. Can I measure the GPDH activity with a spectrophotometer using quartz cuvettes?

Yes. Use a 4:1 ratio of reaction solution to sample for the reaction. Store the samples on ice.

If I measure the activity of the samples with a 96-well microtiter plate, is there a formula for computing the value of the enzyme activity?

The GPDH activity (unit/ml) in the test samples can be calculated from the following formula:

$$\text{GPDH activity (unit/ml)} = \frac{\Delta OD_{340} \times A\ (ml)\ X\ \text{Dilution ratio of the test sample}}{6.22 \times B\ (ml) \times C\ (cm)}$$

ΔOD_{340}: Decrease in the absorbance at 340 nm per minute
A (ml): Total reaction volume
B (ml): The volume of enzyme solution (diluted sample) added
C (cm): Optical path length of the cell used
6.22: Millimolar absorption coefficient of NADH molecules

We calculate the sample height from the area of the bottom of the well and the volume of reaction solution, then simply insert that height value for the length of the optical path into the formula and calculate the number of enzyme units. (The 96-well plate that is supplied with the kit is made by Corning. You can calculate the approximate activity using 0.32 cm^2 as the area of the bottom of the plate.) In addition, when you want to calculate the enzyme activity with precision, determine the correction factor by comparing the OD value measured at 340 nm with a spectrophotometer to that measured with a microtiter plate reader.

If I want to freeze and store samples that were extracted from cells and measure the GDPH activity later, will the enzyme retain full activity after storage?

We measure enzyme activity immediately after preparing the samples because we have found that the activity of crude extract from brown adipocytes decreases after the extract is subjected to a freeze-thaw cycle. Prepared samples remain stable for one hour on ice. If samples must be frozen and stored, divide the samples into small aliquots, maintain them in concentrated form (do not dilute), and store them at –80°C. Avoid repeated freezing and thawing of the samples.

The enzyme reaction did not proceed linearly.

The enzyme concentration may be too high. Use the enzyme extraction buffer to prepare serial dilutions, then measure the enzyme activity of the dilutions to obtain a linear reaction curve.