(Diagnostic Reagent Grade) ASAHI KASEI ENZYMES T-207

Glycerol-3-phosphate dehydrogenase [G3PDHⅡ]

from *Saccharomyces cerevisiae*
(*sn*-glycerol-3-phosphate:NAD^+ 2-oxidoreductase, EC 1.1.1.8)

sn-glycerol 3-phosphate + NAD^+ ↔ glycerone phosphate + NADH + H^+

Preparation and Specification

Appearance	: White lyophilized powder	
Specific activity	: More than 37 U/mg solid	
Contaminants	: NADH oxidase	Less than 0.00015 %(U/U)

Properties

Substrate specificity	: The enzyme is specific for *sn*-glycerol 3-phosphate and NAD(H)	
Molecular weight	: 45.6 kDa (SDS-PAGE)	
Isoelectric point	: pH 4.8-5.3	
Michaelis constant	: *sn*-glycerol 3-phosphate 1.4×10^{-2} M (8.5 mM NAD)	
	: NAD 4.6×10^{-4} M (75 mM *sn*-glycerol 3-phosphate)	
Optimum pH	: 9.0	Figure 1
pH stability	: 6.0–7.0 (37℃, 60 min)	Figure 2
Optimum temperature	: 40℃	Figure 3
Thermal stability	: Stable at 45℃ and below (pH 6.5, 30 min)	Figure 4
Inhibitor[1)]	: ATP, ADP, fructose 1,6-bisphosphate	

Applications for Diagnostic Test

The enzyme is useful for enzymatic determination of **triglyceride (TG)** coupled with LP (Lipase; T-01, T-63, or T-116) and GK (Glycerol kinase; T-64 or T-223).

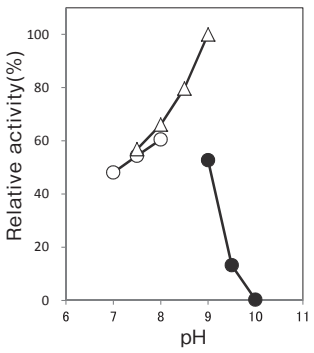
$$\text{TG} + 3H_2O \xrightarrow{\text{LP}} \text{Glycerol} + 3\ \text{Fatty acid}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{GK}} \text{G-3-P} + \text{ADP}$$

$$\text{G-3-P} + NAD^+ \xrightarrow{\textbf{G3PDHII}} \text{DHAP} + \text{NADH} + H^+$$

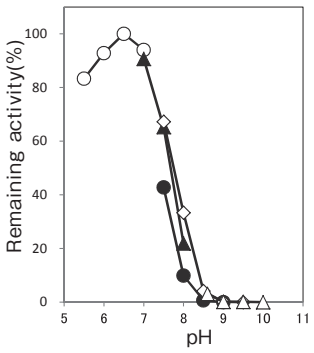
G-3-P: *sn*-Glycerol 3-phosphate
DHAP: Dihydroxyacetone phosphate

Fig.1 pH Optimum



○ : TES-NaOH buffer
△ : Bicine-NaOH buffer
● : CHES-NaOH buffer

Fig.2 pH Stability



37℃, 60 min
0.1M buffer containing 0.1% BSA
○ : MES-NaOH buffer
▲ : TES-NaOH buffer
● : Bicine-NaOH buffer
◇ : Tris-HCl buffer
△ : CHES-NaOH buffer

Fig.3 Optimum Temperature

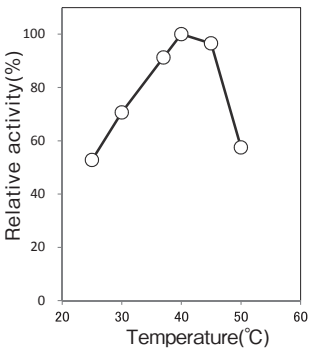
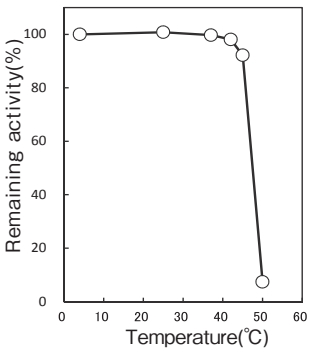


Fig.4 Thermal stability



pH 6.5, 30 min
50mM Phosphate buffer
containing 0.1% BSA

G

Assay

■ Principle

This assay is based on the increase in absorbance at 340 nm as the formation of NADH proceeds in the following reactions:

sn-glycerol 3-phosphate + NAD^+ $\xrightarrow{\text{G3PDH II}}$ glycerone phosphate + NADH

NAD: Nicotinamide adenine dinucleotide

■ Unit definition

One unit is defined as the amount of enzyme which generates 1 μmole of NADH per minute at 37 ℃ under the conditions specified in the assay procedure.

■ Reagents

1. Reaction mixture
   Combine 1.632 g of Bicine with about 70 ml of distilled water, and adjust pH to about 8.5 at about 25 ℃ with 1N NaOH (Bicine will dissolve after adjusting pH). Add 4.727 g of Disodium Glycerophosphate 5.5-hydrate and 0.564 g (based on pure product) of NAD, dissolve them, add distilled water to make up to about 90 ml, and adjust pH to 8.7 at 25 ℃ with 1N NaOH. Finally add distilled water to make up to 100 ml.
2. Enzyme dilution buffer
   Dissolve 1.211 g of tris (hydroxymethyl) aminomethane with 800 ml of distilled water, and adjust pH to 7.5 at 25 ℃ with 1N HCl. Add 1 g of BSA, dissolve it, check the pH of the solution again, and add distilled water to make 1 L solution.
3. Reagent
   Bicine: Dojindo Laboratories #347-03282
   Disodium Glycerophosphate 5.5-hydrate:
   FUJIFILM Wako Pure Chemical Corporation
   Special grade #192-02055
   Tris (hydroxymethyl) aminomethane:
   Sigma Chemical Co. #T-1503
   BSA: Millipore Fraction V pH 5.2 # 81-053
   NAD: FUJIFILM Wako Pure Chemical Corporation
   #304-50443

■ Enzyme solution

Accurately weigh about 20 mg of the sample and add enzyme dilution buffer to make a total of 20 ml. Dilute it with enzyme dilution buffer to adjust the concentration as required.

■ Procedure

1. Pipette accurately 2.00 ml of reaction mixture into a small test tube and preincubate at 37 ℃.
2. After 5 min. add accurately 20 μl of enzyme solution and mix to start the reaction at 37 ℃.
   ※ In the case of a test blank, add 20 μl of enzyme dilution buffer in place of enzyme solution.
3. After starting the reaction, measure the rate of increase per minutes in absorbance at 340 nm. The rate must be measured within the linear portion of the absorbance curve.

   Absorbance sample : As/min
   blank : Ab/min
   ΔA/min = (As/min − Ab/min) ≦ 0.15 Abs/min

■ Calculation

$$\text{Activity (U/mg of powder)} = \frac{\Delta A/\text{min}}{6.22} \times \frac{2.02}{0.02} \times \frac{1}{X}$$

6.22 : millimolar extinction coefficient of NADH at 340 nm (cm^2 / μmole)
2.02 : final volume (ml)
0.02 : volume of enzyme solution (ml)
X : concentration of the sample in enzyme solution (mg/ml)

Storage

Storage at −20 ℃ in the presence of a desiccant is recommended.

References

1. Albertyn J., van Tonder A., Prior BA. (1992) FEBS Lett., 308(2), 130-132.**72**, 972.
2. Miura, M., *et al.,* (1989) J. Clin. Lab. Inst. Reag., **12(5),**1005-1009.

G3PDH Ⅱ活性測定法

Ⅰ．試薬液

1. 反応試薬混合液

Bicine 1.632 g を精製水約 70 ml に混合し、1N NaOH で約 pH8.5（約 25℃）に調整する（pH 調整すると Bicine は溶解する）。さらにグリセロりん酸二ナトリウム 5.5 水和物 4.727 g と NAD 0.564 g を加えて溶解したあと、精製水を加えて全容を約 90 ml とし、1N NaOH で pH8.7（25℃）に調整する（NAD は純度換算する）。最後に精製水で全容を 100 ml とする。

2. 酵素溶解希釈用液

トリス（ヒドロキシメチル）アミノメタン 1.211 g を精製水 800ml に溶解した後、1N HCl で pH7.5（25℃）に調整し、BSA を 1 g 加えて溶解し、再度 pH を確認した後、精製水で 1 L とする。

3. 試薬

Bicine（ビシン）：同仁化学製 #347-03282
グリセロりん酸二ナトリウム 5.5 水和物：
富士フイルム和光純薬製 特級 #192-02055
トリス（ヒドロキシメチル）アミノメタン：
シグマ社製 #T-1503
BSA：Millipore 社製 Fraction V pH5.2 #81-053
NAD（ニコチンアミドアデニンジヌクレオチド・酸化型）：
富士フイルム和光純薬製 #304-50443

Ⅱ．酵素試料液

検品約 20 mg を精密に量り、酵素溶解希釈用液で溶解して全容 20 ml とする。
その液を酵素溶解希釈用液で適宜希釈する。

Ⅲ．測定操作法

1. 小試験管に反応試薬混合液 2.00 ml を正確に分注し、37℃で予備加温する。
2. 5分経過後、酵素試料液 20 μlを正確に加えて混和し、37℃で反応を開始する。
※盲検は酵素試料液の代わりに酵素溶解希釈用液 20 μl を加える。
3. 反応開始後、340 nmにおける吸光度を測定して直線的に反応している1分間当たりの吸光度変化を求める。
求められた吸光度変化を試料液についてはAs/min、盲検液については Ab/min とする。
ΔA/min =（As/min − Ab/min）≦ 0.15 Abs/min

Ⅳ．計算

$$\text{活性 (U/mg)} = \frac{\Delta A/\text{min}}{6.22} \times \frac{2.02}{0.02} \times \frac{1}{X}$$

6.22 : NADH の 340nm におけるミリモル分子吸光係数（$cm^2/\ \mu mol$）
2.02 : 反応総液量（ml）
0.02 : 反応に供した酵素試料液量（ml）
X　: 酵素試料液中の検品濃度（mg/ml）