Malate Dehydrogenase“Amano”3

((*S*)-Malate : NAD^+ oxidoreductase, EC 1.1.1.37)

Malate Dehydrogenase“Amano”3 is a malate dehydrogenase preparation, manufactured using recombinant bacteria.

Catalysis

L-Malate + NAD^+ ⇄ Oxaloacetate + NADH + H^+

Specification and Preparation

Activity:	Malate dehydrogenase activity	≧ 100 u/mg (Amano method)
Contaminants:	NADH oxidase activity	≦ 4×10^{-4}%
	α-KG dehydrogenase activity	≦ 3×10^{-3}%
	GOT activity	≦ 3×10^{-3}%
Appearance:	White powder, lyophilized	
Additive:	Dextran	

Characteristics

1. Molecular weight: 35,000 (SDS-PAGE)
2. Isoelectric point: 4.8
3. Km: 5.6×10^{-5} M (Oxaloacetate)
   6.6×10^{-5} M (NADH)
4. Optimum pH: 7.8
5. pH stability: 8.0-9.0 (70°C, 30 min)
6. Optimum temperature: 70°C
7. Thermal stability: up to 80°C (pH 8.5, 30 min)
8. Inhibitor: Zn^{2+}
9. Application: Used for the enzymatic determination of L-malate and aspartate aminotransferase (AST) in clinical diagnosis.

Expiration (Storage)

18 months from the date of analysis when stored at 5°C or below in a dry place under sealed conditions.

The information and recommendations contained herein are to the best of our knowledge reliable according to the current scientific and technical level. However, depending upon use method and/or condition, nothing herein is to be construed as a warranty or representation in respect otherwise, including freedom from patent infringement. Users shall make their own test and investigation for their particular purpose. We do not accept any liability for any loss, damage or infringement arising from the use of information and recommendations contained herein.

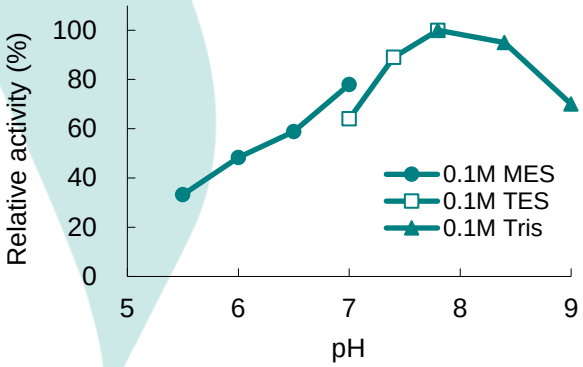
Safe Handling

1. Do not inhale.
2. In case of direct contact with skin or eyes, immediately wash or rinse with plenty of water.
3. Please refer to SDS for more details.

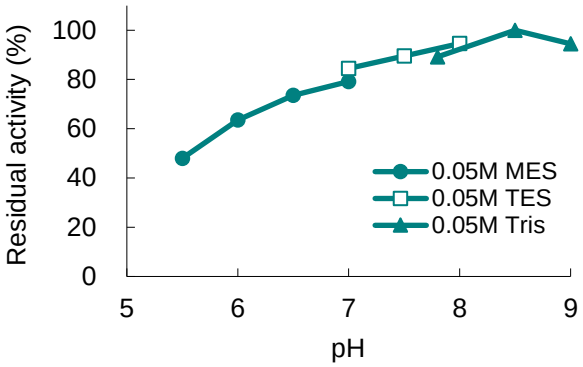
General properties

The following results demonstrate the activities of enzyme solution prepared in various buffers. Enzyme activity may vary under different experimental conditions.

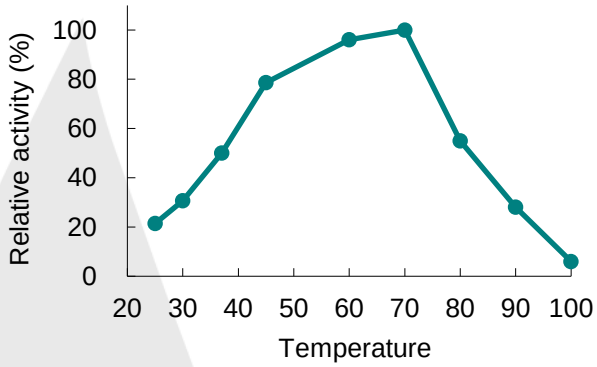
pH and Activity



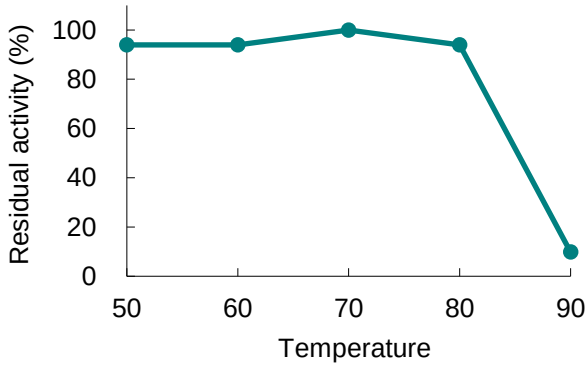
pH stability



Temperature and Activity



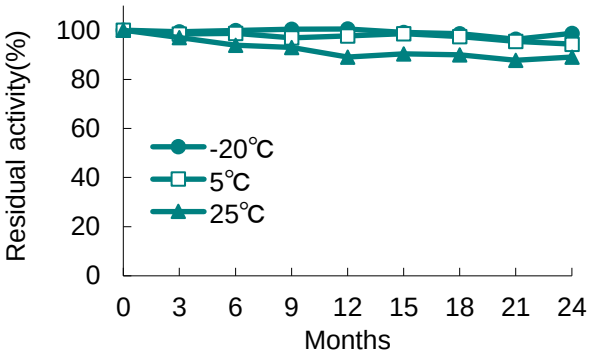
Thermostability



Effect of Various Chemicals

Chemicals	Concentration (mM)	Relative Activity (%)
None	—	100
$MnCl_2$	1.0	97
$MgCl_2$	1.0	95
$ZnCl_2$	1.0	57
$CuCl_2$	1.0	88
EDTA	1.0	89
PCMB	1.0	92
MIA	1.0	92
NaN_3	1.0	92

Stability (powder form)



Assay method of Malate dehydrogenase activity

Principle

$$\text{Oxaloacetic acid} + \text{NADH} + \text{H}^+ \xrightarrow{\text{Malate Dehydrogenase}} \text{L-Malic acid} + \text{NAD}^+$$

The consumption of NADH is measured at 340 nm by spectrophotometry.

Unit Definition

One unit is defined as the enzyme quantity which oxidizes one μ mole of NADH per minute under the conditions described below.

Reagents

A. 0.1 M Tris-HCl buffer (pH7.8)
B. 0.01 M Phosphate buffer (KH_2PO_4-NaOH, pH 7.0)
C. Triton X-100 solution (50 mg/ml)
D. 0.01 M Phosphate buffer containing 0.1% Triton X-100 (KH_2PO_4-NaOH, pH 7.0)
   Dilute 20 ml of Triton X-100 solution (C) with approx. 800 ml of 0.01M Phosphate buffer (B). Fill up to 1,000 ml with 0.01M Phosphate buffer (B).
E. NADH solution
   Weigh 9 mg of NADH (Oriental Yeast Co., Ltd.) and dissolve in 0.1M Tris-HCl buffer (A). Fill up to 50 ml with 0.1M Tris-HCl Buffer (A). (Can be used for 5 days if kept refrigerated)
F. Substrate solution
   Weigh 11 mg of oxaloacetic acid and dissolve in 0.1M Tris-HCl buffer (A). Fill up to 50 ml with 0.1M Tris-HCl buffer (A). (Make a fresh solution for each use.)
G. Enzyme solution
   Weigh out Malate Dehydrogenase“Amano”3 and dissolve in chilled 0.01M Phosphate Buffer containing 0.1% Triton X-100 (D). Enzyme solution should be prepared so that the value of ΔOD/minute becomes in the range of 0.025 ± 0.010.

Procedure

Pipette 2.0 ml of NADH solution (E) and 0.90 ml of Substrate solution (F) respectively into a quartz cell (d=10 mm) and keep at 25 ± 0.5℃ for 5 minutes. Then, pipette 0.10 ml of Enzyme solution (G) into the quartz cell and mix well immediately. Keep the reaction mixture at 25 ± 0.5℃.
Exactly at 2 minutes and 5 minutes after the addition of Enzyme solution (G), measure the absorbances of the reaction mixture at 340 nm (A2 and A5).
As a blank, pipette 0.01M Phosphate buffer (D) into another quartz cell (d=10 mm) instead of the Enzyme solution (G) and follow the same procedure described above (Ab2 and Ab5).

Calculation

$$\text{Malate dehydrogenase activity (u/mg)} = \frac{(A2-A5)-(Ab2-Ab5)}{3} \times \frac{1}{6.22} \times 3.0 \times \frac{n}{0.1}$$

3: Reaction time
6.22: Millimolar absorption coefficient of NADH at 340 nm
3.0: Volume of the reaction mixture
0.1: Volume of Enzyme solution
n: Dilution factor of Enzyme solution

Contact

Area	Branch	Location	E-mail
North, Central, South America	Amano Enzyme U.S.A. Co., Ltd.	Illinois,U.S.A.	aeu.sales@amano-enzyme.com
Europe, the Middle East and Africa	Amano Enzyme Europe Limited	Oxfordshire, U.K.	aee.sales@amano-enzyme.com
Asia Pacific	Amano Enzyme Asia Pacific Co., Ltd.	Pathum Thani, THAILAND	aeap.sales@amano-enzyme.com
China	Amano Enzyme Manufacturing (China), Ltd. Shanghai Branch	Shanghai, P.R.CHINA	shanghai@amano-enzyme.com.cn
Japan, Headquarters	Amano Enzyme Inc.	Nagoya, JAPAN	