3-Hydroxybutyrate Dehydrogenase“Amano”5

((R)-3-Hydroxybutanoate: NAD^+ oxidoreductase, EC 1.1.1.30)

3-Hydroxybutyrate Dehydrogenase“Amano”5 is a D-3-hydroxybutyrate dehydrogenase preparation, manufactured using recombinant bacteria.

Catalysis

D-3-Hydroxybutyrate + NAD^+ $\rightleftharpoons$ Acetoacetate + NADH + H^+

Specification and Preparation

Activity:	HBDH activity	≧ 75 u/mg (Amano method)
Appearance:	White to light brown powder, lyophilized	
Additive:	Not added	

Characteristics

1. Molecular weight: 27,000 (SDS-PAGE)
   75,000-80,000 (Gel filtration)
2. Isoelectric point: 4.8
3. Km: 5.0×10^{-4} M (D-3-Hydroxybutyrate)
   4.2×10^{-5} M (NAD^+)
4. Optimum pH: 8.5
5. pH stability: 6.0-10.0 (37°C, 1hr)
6. Optimum temperature: 55°C
7. Thermal stability: up to 37°C (pH 7.0, 1hr)
8. Inhibitors: Fe^{2+}, *N*-Ethylmaleimide
9. Application: Used for the enzymatic determination of ketone body (D-3-Hydroxybutyrate or Acetoacetate) in plasma in clinical diagnosis.

Expiration (Storage)

36 months from the date of analysis when stored at -20°C or below in a dry place under sealed conditions.

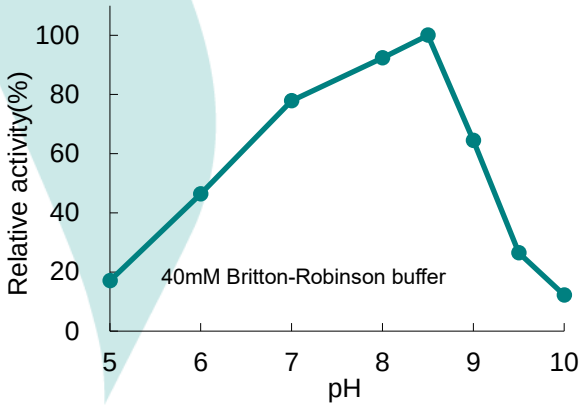
Safe Handling

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.

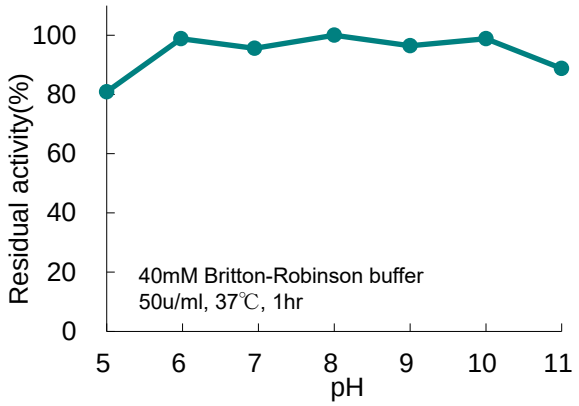
General properties

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

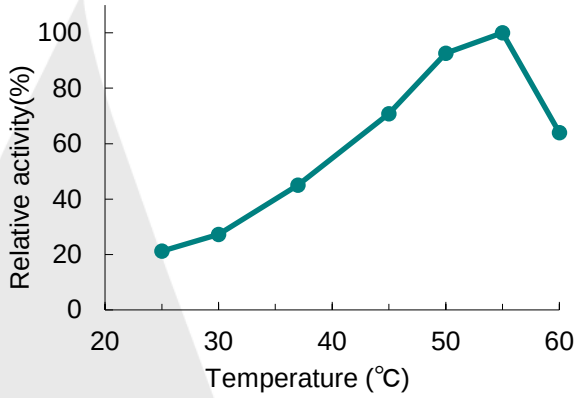
pH and Activity



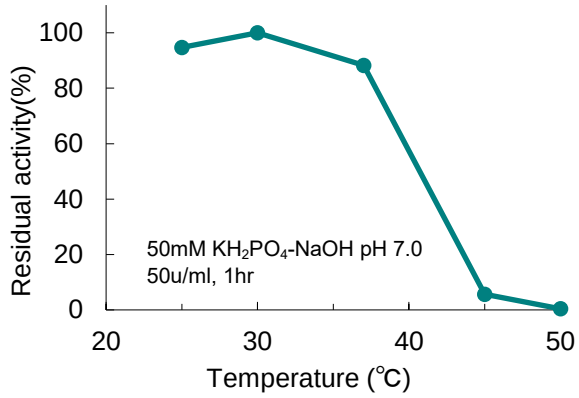
pH stability



Temperature and Activity



Thermostability



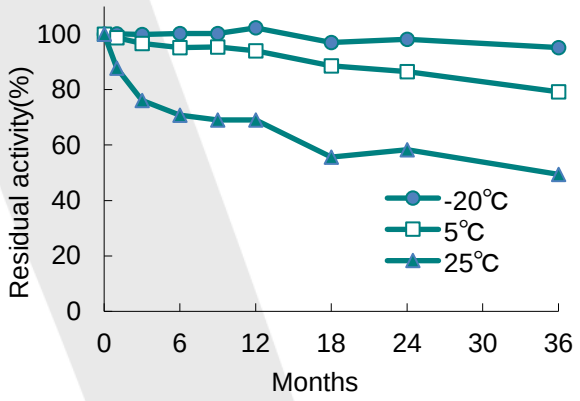
Substrate Specificity

Substrate	Relative Activity (%)
3-Hydroxybutyrate	100
2-Hydroxybutyrate	0
Lactate	0
Glycerate	0
L-Malate	0
DL-Malate	0
n-Butanol	0
Glycolate	0
NAD^+	100
$NADP^+$	0

Effect of Various Chemicals

Chemical	Concentration (mM)	Relative Activity (%)
None		100
NaF	2	104
NaN_3	20	104
o-Phenanthroline	2	96
α, α'-Dipyridyl	1	100
Boric acid	50	96
N-Ethylmaleimide	2	48
Hydroxylamine	2	92
$ZnCl_2$	2	100
$BaCl_2$	2	92
$CaCl_2$	2	92
$MgCl_2$	2	98
$MnCl_2$	2	96
$NiCl_2$	2	100
$CoCl_2$	2	98
$FeSO_4$	2	77
$CuSO_4$	2	94

Stability (powder form)



Assay method of D-3-hydroxybutyrate dehydrogenase (HBDH) activity

Principle

D-3-hydroxybutyrate + NAD^+ —(D-3-Hydroxybutyrate dehydrogenase)→ Acetoacetate + NADH + H^+

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

Unit Definition

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

Reagents

A. 0.1M Tris-HCl buffer (pH8.5)
B. Hydrazine sulfate solution
Weigh 75 mg of Hydrazine Sulfate and dissolve in 0.1M Tris-HCl Buffer (A), then adjust to pH 8.5 with 4N NaOH. Fill up to 100 mL with 0.1M Tris-HCl Buffer (A). (Make a fresh solution for each use.)
C. Triton X-100 stock solution (5g/dL deionized water)
D. Substrate solution
Weigh 151.2 mg of (+/-)-3-Hydroxybutyric acid sodium salt (FUJIFILM Wako Pure Chemical Corporation) and dissolve in 10 mL of deionized water. (Make a fresh solution for each use.)
E. NAD solution
Weigh 86.1 mg of β-NAD^+ (Oriental Yeast Co., Ltd.) and dissolve in 10 mL of deionized water. (Make a fresh solution for each use.)
F. Diluent (0.1M Tris-HCl buffer (pH 8.5) containing 0.1% Triton X-100 and 0.1% BSA)
Weigh 12.1 g of Tris (hydroxymethyl) aminomethane and 1.0 g of BSA (FIJIFILM Wako Pure Chemical Corporation, Law salt), dissolved in approx. 800 ml of deionized water. Add 20 ml of Triton X-100 stock solution (C), then adjust to pH 8.5 with 4N HCl. Fill up to 1L with deionized water. (Can be used for 14 days if kept refrigerated)
G. Enzyme solution
Weigh out 3-Hydroxybutyrate Dehydrogenase“Amano”5 and dissolve in chilled Diluent (F). Enzyme Solution should be prepared so that the value of ⊿OD/minute becomes in the range of 0.040±0.010.

Procedure

Pipette 2.0 ml of Hydrazine sulfate solution (B), 0.5 ml of Substrate solution (C) and 0.5 ml of NAD solution (E) respectively into a quartz cell (d =10 mm) and keep at 25±0.5℃ for 5 minutes. Then, pipette 0.05 ml of Enzyme solution (G) into the quartz cell and mix well immediately. Keep the reaction mixture at 25±0.5℃. Exactly at 5 minutes and 8 minutes after the addition of Enzyme solution (G), measure the absorbances of the reaction mixture at 340 nm (A5 and A8). As a blank, pipette Triton X-100 solution (F) into another quartz cell (d =10 mm) instead of Enzyme solution (F) and take the same procedure described above (Ab5 and Ab8).

Calculation

$$\text{D-3-Hydroxybutyrate dehydrogenase activity (u/mg)} = \frac{(A8-A5)-(Ab8-Ab5)}{3} \times \frac{1}{6.22} \times 3.05 \times \frac{n}{0.05}$$

3: Reaction time
6.22: Millimolar absorption coefficient of NADH at 340 nm
3.05: Volume of the reaction mixture
0.05: Volume of Enzyme Solution
n: Dilution factor of Enzyme Solution

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