

SPECIFICATION SHEET

Product Name: Diaphorase
Catalog # DA01D1-E311H

Product Description: This enzyme is a diagnostic grade reagent from a microorganism host. It is useful for enzymatic determination of reduced NAD.

EC 1.6.5.2

Storage and Stability: Product can be stored at 2~8°C for transportation process up to ten days but long-term storage should be at -20°C.

Principle:

$\text{NAD(P)H} + \text{H}^+ + \text{Acceptor (ox)} \xrightarrow{\text{acceptor oxidoreductase}} \text{NAD(P)}^+ + \text{Acceptor}$

Unit Definition	: One unit causes the decrease of one micromole of DCPIP per minute at 37°C under the conditions specified in the assay procedure.	
Appearance	: Yellowish amorphous powder, lyophilized	
Activity	: ≥100 U/mg solid	
Contaminants	: Myokinase	≤5.0 × 10 ⁻¹ %
	: NADH oxidase	≤1.0 × 10 ⁻¹ %
Molecular Weight	: 26.3 kDa	
Isoelectric Point	: 7.72	
Michaelis Constant	: 2.23 × 10 ⁻¹ M (NADH), 5.72 × 10 ⁻¹ M (NADPH)	
Inhibitors	: Fe ³⁺ , Cu ²⁺ , Hg ²⁺ , Ag ⁺	
Optimum pH	: 6.0, 8.0-10.0	(Figure 1)
Optimum Temperature	: 40~45°C	(Figure 2)
pH Stability	: pH 6.0~11.0 (25°C, 20 hr)	(Figure 3)
Thermal Stability	: below 62°C (pH 7.5, 15 min)	(Figure 4)
Substrate Specificity	: (Table 1)	
Effect of Various Chemicals	: (Table 2)	

Table 1. Substrate Specificity of Diaphorase

Substrate	Residual Activity (%)
NAPH	100
NADPH	1.5

Table 2. Effect of Various Chemicals on Diaphorase

The enzyme dissolved in 0.1 M HEPES buffer, pH 7.5 (5.0 U/mL) was incubated with each chemical at 25°C for 1 hr.

Chemical	Conc. (mM)	Residual activity (%)	Chemical	Conc. (mM)	Residual activity (%)
None	—	100.0	BME	2	98.5
CaCl ₂	2	99.6	Hydroxylamine	2	91.0
MgSO ₄	2	100.9	EDTA	5	92.0
ZnSO ₄	2	73.3	NaF	20	94.9
NiCl ₂	2	81.8	NaN ₃	20	111.5
CoCl ₂	2	79.5	Proclin-300	0.045% (v/v)	85.2
MnCl ₂	2	72.5	SDS	0.05% (w/v)	14.5

FeCl₃ 2 25.8

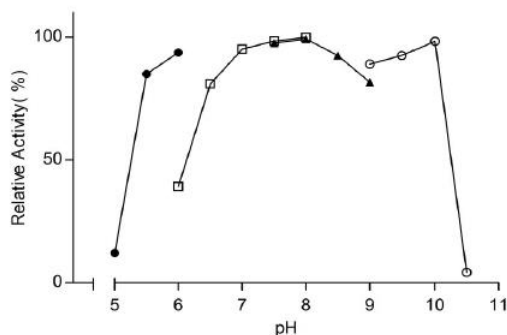
Na-Cholate 0.1% (w/v) 98.3

Table 2 (Continued)

Chemical	Conc. (mM)	Residual activity (%)
CuSO ₄	2	0.0
AgNO ₃	2	24.2
HgSO ₄	2	14.0
NEM	2	89.1

Chemical	Conc. (mM)	Residual activity (%)
Tween-20	0.1% (v/v)	94.1
Triton X-100	0.1% (v/v)	89.0
Span-20	0.1% (v/v)	81.1
Brij-35	0.1% (v/v)	90.8

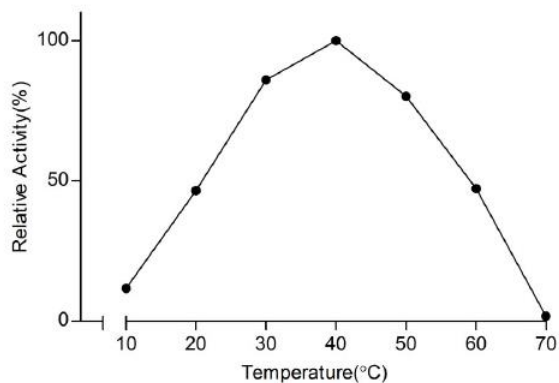
Fig.1. pH Activity



37°C in the following buffer solution:

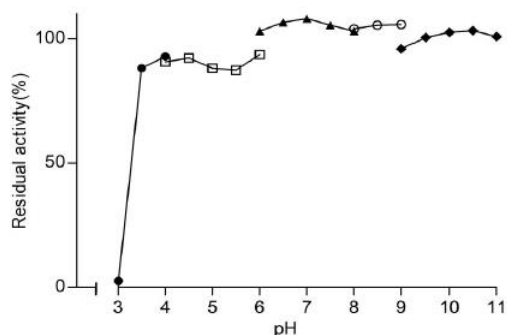
- 0.1M Acetate buffer
- 0.1M K-phosphate buffer
- ▲ 0.1M Tris-HCl buffer
- 0.1M Glycine-NaOH buffer

Fig.2. Temperature Activity



in 50mM K-phosphate buffer, pH 7.5

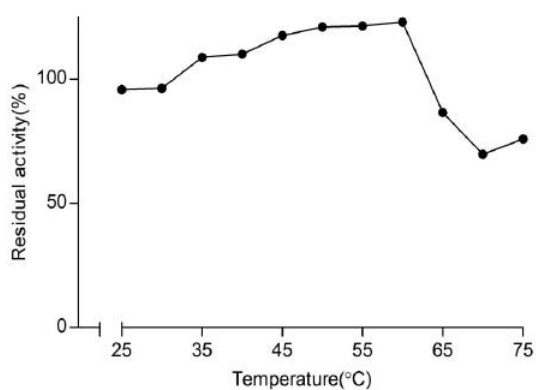
Fig.3. pH Stability



25°C, 20hr-treatment with following buffer solution:

- 0.1M Glycine-HCl buffer
- 0.1M Acetate buffer
- ▲ 0.1M K-phosphate buffer
- 0.1M Tris-HCl buffer
- ◆ 0.1M Glycine-NaOH buffer

Fig.4. Thermal Stability



15min- treatment with 0.1M K-phosphate buffer, pH7.5

