Recombinant Diaphorase (DIA)

D776907

Reaction

$$NAD(P)H + H^{+} + Acceptor(ox) \xrightarrow{DIA} NAD(P)^{+} + Acceptor(red)$$

Product description

A flavoprotein. The enzyme catalyses a two-electron reduction and has a preference for short-chain acceptor quinones, such as ubiquinone, benzoquinone, juglone and duroquinone. The animal, but not the plant, form of the enzyme is inhibited by dicoumarol.

Appearance: Yellowish amorphous powder.

powder Source: Microorganism.

Enzyme Commission Number: EC 1.6.5.2.

CAS Number: 9032-20-6.

Specific activity: ≥20U/mg enzyme powder; ≥35U/mg protein.

Unit definition: One unit will convert one micromole of NADH to NAD^+ per min at pH 8.0 at 37℃.

Properties

Stability:	Stable at -20℃ for at least two years	
Molecular weight:	24kD	
Isoelectric point:	6.0	
Michaelis constant:	2.6×10^{-3}M (NADH)	
Optimum pH:	8.0-11.0	{Fig. 1}
Optimum temperature:	40℃-65℃	{Fig. 3}
pH Stability:	7.0-10.0 (25℃, 24hr)	{Fig. 2}
Thermal stability:	< 50℃ (pH 8.0, 30min)	{Fig. 4}
Inhibitors:	Co^{2+}, Cu^{2+}, Fe^{3+}, Mn^{2+}, Ni^{2+}, Zn^{2+}, NEM, SDS	
Effect of various chemicals:		{Table 1}

Table 1.

Effect of Various Chemicals on DIA.

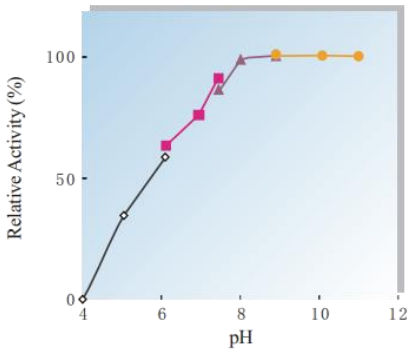
[The enzyme dissolved in 100mM Tris-HCl buffer, pH8.0+0.1%BSA(5U/ml) was incubated

with each chemical at 37℃ for 2hr.]

Chemical	Conc.(mM)	Residual activity
None	-	100%
$CaCl_2$	2.0	88%
$CoCl_2$	2.0	10%
$CuSO_4$	2.0	44%
$FeCl_3$	2.0	28%
$MgSO_4$	2.0	93%
$MnSO_4$	2.0	60%
$NiCl_2$	2.0	46%
$ZnSO_4$	2.0	81%
$K_4Fe(CN)_6$	2.0	100%

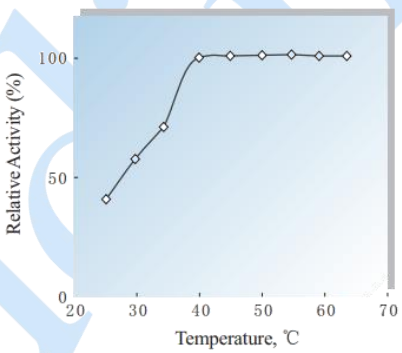
Chemical	Conc.(mM)	Residual activity
BEM	2.0	96%
NEM	2.0	68%
EDTA	5.0	100%
NaN_3	20.0	100%
Proclin	0.045%	100%
Na-cholate	0.10%	88%
SDS	0.05%	42%
Triton X-100	0.10%	100%
Tween 20	0.10%	100%

Fig. 1 pH Activity



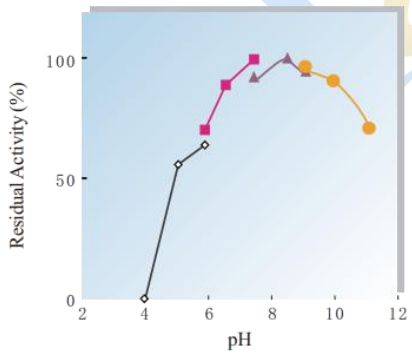
◇: 100mM acetate buffer
■: 100mM phosphate buffer
▲: 100mM Tris-HCl buffer
●: 100mM Glycine-NaOH buffer

Fig. 3 Temperature activity



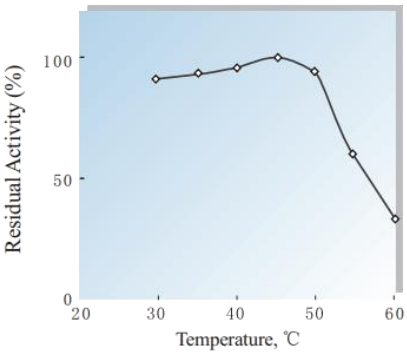
Buffer: 100mM phosphate buffer, pH 8.0

Fig. 2 pH Stability



Treatment : 25℃,20hr
◇: 100mM acetate buffer
■: 100mM phosphate buffer
▲: 100mM Tris-HCl buffer

Fig. 4 Thermal stability



Treatment: 100mM phosphate buffer, pH 8.0, 30min