

Diaphorase (EC 1.8.1.4), *Escherichia coli*

Catalogue number:

AE00231, 1000 U (69 mg)

Description

Diaphorase or dihydrolipoyl dehydrogenase (EC 1.8.1.4) is a flavoprotein enzyme capable of oxidizing the reduced form of NAD (NADH). This lipoamide dehydrogenase is a component of the glycine cleavage system, as well as of the alpha-ketoacid dehydrogenase complexes. It binds one FAD per protein subunit.

The enzyme is provided in 3.2 M ammonium sulphate.

For assay, this enzyme should be diluted in 0.5 mM FAD containing 0.5 mg/mL BSA.

Purity

Diaphorase has been determined to be >95% pure, according to SDS polyacrylamide gel electrophoresis (PAGE) followed by Coomassie Blue staining (Figure 1).

Storage temperature

Diaphorase should be stored at 2°C to 8°C.

Specific activity

14.5 U/mg protein, 174 U/ml.

Unit definition

One Unit of diaphorase was defined as the amount enzyme required to produce 1 μ mole of NAD⁺ from NADH, at 25 °C and pH 9.0.

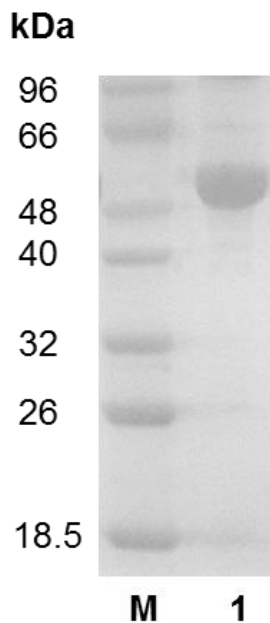


Figure 1. SDS-PAGE analysis of *E. coli* diaphorase. Electrophoresis was performed using a 12% polyacrylamide gel. Lane M, molecular weight marker; Lane 1, purified diaphorase (51 kDa).

Certificate of Analysis

Test	Criteria	Result
Protein purity	Purity in line with the stated value	Meets specification
Protein concentration	Concentration in line with the stated value	Meets specification
Catalytic activity	Activity in line with the stated value	Meets specification
Blank assay variability	Absorbance values with less than 10% of variability	Meets specification

Approved by:



Patricia Ponte
Senior Manager, Quality Systems

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