



## Lactate Dehydrogenase from *Sus scrofa cristatus*

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**Abstract:** Lactate dehydrogenase, LDH, (*EC. 1.1.1.27*), an anaerobic glycolysis enzyme present in all vertebrates, was isolated from the skeletal and heart muscle tissues of domestic pig, *Sus scrofa cristatus*. The process of purifying LDH from porcine tissue samples entailed the use of techniques like centrifugation, ammonium sulfate precipitation, dialysis, and gel filtration chromatography. At each stage of purification, enzyme and protein concentration assays were applied to each fraction collected in order to determine the degree of purification. The enzyme activity was determined by monitoring the rate of conversion of  $\text{NAD}^+$  to NADH as lactate is converted to pyruvate, while protein concentration was determined using the Bradford Assay. The values of the Michaelis-Menten constant ( $K_m$ ), the catalytic constant ( $K_{cat}$ ), and catalytic efficiency ( $K_{eff}$ ) were determined using the Michaelis-Menten, Lineweaver-Burk, Eadie-Hofstee, and Hanes-Woolf plots. These kinetic parameters were used to evaluate LDH in terms of binding and catalysis with respect to the coenzyme  $\text{NAD}^+$  in presence of the other substrate used in the assay, which is lactate. Results show that the  $K_m$  values of skeletal muscle LDH was lower as compared to that of the heart isozyme implying a higher affinity for  $\text{NAD}^+$ . On the other hand the heart LDH isozyme showed a higher  $K_{cat}$  value while the skeletal muscle LDH isozyme has a higher  $K_{eff}$ . The isolates were also characterized using sodium dodecyl polyacrylamide gel electrophoresis (SDS PAGE). The subunits of this tetrameric enzyme were seen as distinct bands around 36,000 Daltons. This study is intended to establish an alternative source of lactate dehydrogenase for both teaching and research laboratories.

**Key Words:** lactate dehydrogenase; gel filtration; SDS-PAGE; isozymes;  $K_m$ ;  $K_{cat}$ ;  $K_{eff}$