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## Immobilization of alkaline protease PersiProtease1 by CLEA method

Yashar Najafi, Aysuda Mohamadzadeh, Manijeh Sabokdast Nodehi\*, Shohreh Ariaeenejad\*

- 1. Department of Agronomy and Plant breeding, College of Agriculture & Natural Resources, University of Tehran, Karaj, Iran, yasharnajafi7815@ut.ac.ir
- 2. Department of Cell and Molecular Biology, Faculty of Biological Sciences, Kharazmi University, Tehran, Iran
- 3. Department of Agronomy and Plant breeding, College of Agriculture & Natural Resources, University of Tehran, Karaj, Iran
- 4. Department of Systems and Synthetic Biology, Agricultural Biotechnology Research Institute of Iran (ABRII), Karaj, Iran

## Abstract

Proteases are essential enzymes extensively employed in numerous industrial processes, including food production, detergent manufacturing, pharmaceuticals, and leather processing, where they play a pivotal role in enhancing efficiency, product quality, and sustainability by facilitating the breakdown of proteins under diverse conditions. Nonetheless, the industrial use of proteases is constrained by challenges such as reduced stability under harsh conditions, high production costs, and difficulties in enzyme recovery and reuse, which hinder their long-term and cost-effective application in various sectors. Enzyme immobilization has proven to be a viable strategy to address many of these challenges. Among different immobilization techniques, the Cross-Linked Enzyme Aggregates (CLEA) method stands out for enhancing enzyme stability through cross-linking enzyme molecules with bifunctional agents, without the need for external carriers or coatings. In this study, the alkaline protease PersiProtease1 was immobilized using the CLEA method. The immobilization involved using 55% (w/v) saturated ammonium sulfate as the precipitant and 25% (w/v) glutaraldehyde as the cross-linker, maintaining the reaction at 25°C for 17 hours. The immobilized enzyme exhibited a stability increase of over two-fold compared to the free enzyme, demonstrating the CLEA method's efficacy in enhancing enzyme performance. These findings highlight promising applications in biodiesel production, wastewater treatment, textile and leather processing, detergent formulation, and pharmaceutical industries.

**Key words:** Cross-Linked Enzyme Aggregates, Enzyme Immobilization, Glutaraldehyde, Industrial Application, Protease





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