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Efficient production of highly purified Late Embryogenesis Abundant (LEA) protein from *Arabidopsis thaliana* by recombinant DNA technology

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Late embryogenesis abundant (LEA) proteins are induced in cellular dehydration, such as freezing, drought, or desiccation. They can be involved in antioxidative defense, ion sequestration, and structural stabilization of both membranes and enzymes during freezing or drying, while by forming intracellular proteinaceous condensates they increase structural integrity and intracellular viscosity of cells during desiccation [1]. The genome of the model plant *Arabidopsis thaliana* contains 51 genes encoding LEA proteins[2]. The majority of these LEA proteins (35%) belongs to Pfam LEA_4 (PF02987) family. *In silico* analysis suggested that these proteins are highly hydrophilic proteins with significant intrinsically disordered protein (IDP) properties. In order to evaluate structural properties and possible functions of LEA_4 protein family under different water content, a representative AtLEA25 protein (At2g42560, 635 aa), naturally located in the cytoplasm of seeds[3] was obtained in *Escherichia coli* by recombinant DNA technology. Although this technology has been traditionally used to over-express and purify various globular proteins, numerous reports have shown that the IDPs, due to their structural plasicity are naturally highly susceptible to proteolytic cleavage. To conduct structural and functional studies we developed a robust method to produce highly purified (>95% pure) AtLEA25 with no detectable amount of protein breakdown products.

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