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Enhanced expression and purification strategy for recombinant bacterially - expressed human hexokinase II

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Abstract

Author keywords

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Funding details

Abstract

Dengue virus hijacks the host cellular mechanism to propagate and survive during viral infection, in which the central carbon mechanism plays a crucial role to upregulate DENV infection through the increase of human hexokinase II (HKII) activity. Since the enzyme governs the glycolytic pathway, it has potentials as a target for anti-dengue (DENV) drug development. In this study, the production of human hexokinase II protein has been enhanced by using bacterial system for anti-dengue therapeutic purpose. The HKII gene was cloned into pET28b vector and transformed into the E. coli strain BL21 (DE3) for HKII expression . In order to obtain soluble recombinant HKII in an active form, we optimized protein expression under specific conditions at 18 °C for 19 hours using Terrific Broth media, in the presence of 0.5 mM isopropyl-2-D-thiogalactopyranoside (IPTG). The pET28b-HKII construct expressed in BL21 (DE3) system exhibited adequate protein expression , thus, this construct was subsequently proceeded to purification process. The expressed protein was purified to homogeneity by a combination of Immobilized Metal Ion Affinity Chromatography (IMAC) and size exclusion chromatography (SEC), resulting in pure, active bacterially - expressed HKII with a specific activity of 80.90 $U.mg^{-1}$. The amount of HKII obtained from 2 L culture is 80 mg, with a yield percentage of 10.50%. Hence in this study, human HKII has successfully been cloned and expressed as a soluble protein that can be utilized for further therapeutic studies.

Author keywords
Gene cloning; Glycolysis; Human hexokinase II protein expression ; Protein purification

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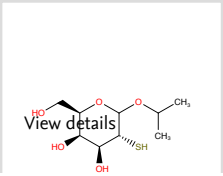

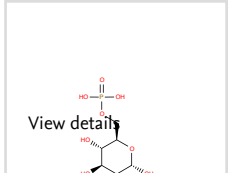

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