



Purification of Small Structural Protein of Chikungunya Virus-6K

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Keywords

CHIKV; 6K protein; pGEX-4T3; Glutathione affinity column chromatography; Thrombin cleavage; GST-6K fusion protein.

ABSTRACT: Chikungunya Virus (CHIKV) is an enveloped, spherical alpha-virus with (+)ssRNA as its genomic content. It encodes both structural (C, 6K, E1, E2, E3) and non structural genes (nsP1-nsP4) along with polyadenylation sites flanking the 5' and 3' ends. This virus causes the Chikungunya disease which is characterized by fever, rashes and anthralgia. This study aims to purify 6K structural protein produced by the virus. The strategy involves cloning of 6K gene into pGEX-4T3 cloning vector for production of the native protein. The 6K gene was already cloned into pGEX expression system in the laboratory. The conditions for the 6K protein expression were optimized and the protein was induced using IPTG. The protein solubilization was optimized with Triton X-100 detergent. Purification was carried out using Glutathione affinity column chromatography. Thrombin cleavage at varied conditions was carried out to obtain the native 6K protein using two separate methods. One of them involved the purification of the recombinant GST-6K fusion protein and then cleavage of the GST tag from the fusion protein to achieve the native 6K protein. The other way was one step process where the 6K protein was cleaved by thrombin protease during purification when the GST-6K fusion protein was attached to the Glutathione beads in the column thereby leaving the GST tag attached to the beads and generation of the native 6K protein. Experimental data showed that varied conditions used for thrombin cleavage were unable to cleave the GST-6K fusion protein. It needs to be further optimized. © 2016 iGlobal Research and Publishing Foundation. All rights reserved.

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