

Purification and Pre-Crystallization of Recombinant PhoR Cytoplasmic Domain Protein from Mycobacterium Tuberculosis H37Rv

Authors : Oktira Roka Aji, Maelita R. Moeis, Ihsanawati, Ernawati A. Giri-Rachman

Abstract : Globally, tuberculosis (TB) remains a leading cause of death. The emergence of multidrug-resistant strains and extensively drug-resistant strains have become a major public concern. One of the potential candidates for drug target is the cytoplasmic domain of PhoR Histidine Kinase, a part of the Two Component System (TCS) PhoR-PhoP in Mycobacterium tuberculosis (Mtb). TCS PhoR-PhoP relay extracellular signal to control the expression of 114 virulent associated genes in Mtb. The 3D structure of PhoR cytoplasmic domain is needed to screen novel drugs using structure based drug discovery. The PhoR cytoplasmic domain from Mtb H37Rv was overexpressed in *E. coli* BL21(DE3), then purified using IMAC Ni-NTA Agarose his-tag affinity column and DEAE-ion exchange column chromatography. The molecular weight of the purified protein was estimated to be 37 kDa after SDS-PAGE analysis. This sample was used for pre-crystallization screening by applying sitting drop vapor diffusion method using Natrix (HR2-116) 48 solutions crystal screen kit at 25°C. Needle-like crystals were observed after the seventh day of incubation in test solution No.47 (0.1 M KCl, 0.01 M MgCl₂·6H₂O, 0.05 M Tris-Cl pH 8.5, 30% v/v PEG 4000). Further testing is required for confirming the crystal.

Keywords : tuberculosis, two component system, histidine kinase, needle-like crystals

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