Kloning ekspresi dan purifikasi protein rekombinan prm e virus dengue serotipe 4 untuk pengembangan kandidat vaksin = Cloning expression and purification of protein recombinant prm e serotype 4 dengue virus to develop candidate vaccine

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Abstrak

Demam Dengue (DD) dan Demam Berdarah Dengue (DBD) adalah penyakit yang tersebar luas. Penelitian ini dilakukan untuk mengembangkan kandidat vaksin dengue nasional berbasis protein sub unit prM/E DEN-4. Protein prM/E adalah kompleks unik yang berperan penting dalam perakitan virus dan modulasi fusi. Penelitian telah dilakukan dengan metode Gateway cloning system untuk menklon gen prM/E dalam plasmid cloning pDONR221 kemudian dilakukan subkloning dan dipindahkan gen prM/E ke dalam plasmid eksperesi pET-55-DEST. Ekspresi protein prM/E dilakukan di dalam E.coli BL21 (DE3) dengan induksi Isoprophyl-β-D-thiogalactopyranoside (IPTG). Pendeteksian poliprotein Gag hasil ekspresi dilakukan dengan metode Sodium Dodecyl Sulphate Polyacrilamide Gel Electrophoresis (SDS-PAGE). Setelah protein prM/E berhasil dideteksi kemudian protein prM/E dipurifikasi dengan menggunakan metode immobilized metal affinity chromatography (IMAC) di bawah kondisi denaturasi. Hasil penelitian yaitu protein prM/E dapat diekspresikan dalam E.coli BL21 (DE3) dengan berat molekul ~75 kDa.
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Dengue Fever is an infectious disease caused by one of the four serotypes of dengue virus (DENV). Until now, no licensed vaccines or antivirus is available commercially. Because of that, this research was aimed to develop candidate vaccine dengue based on protein subunit pre-membrane and Envelope (prM/E). Protein prM/E is a unique complex which has important role in virus assembly
and host cell entry. The recombinant protein development was done using Gateway cloning system. This was used to clone the prM/E gene into pDONR221 plasmid. The cloned gene was then transferred into pET-55-DEST expression plasmid. Expression of protein prM/E was perfomed in E. coli BL21 (DE3) with inducer Isoprophyl-β-D-thiogalactopyranoside (IPTG). Sodium Dodecyl Sulphate Polyacrilamide Gel Electrophoresis (SDS-PAGE) method was used to detect the expressed prM/E protein. Upon detection of prM/E protein with SDS-PAGE, the recombinant protein was purified by using immobilized metal affinity chromatography (IMAC) method under denature condition. Using these methods, the prM/E protein was successfully expressed in E.coli BL21 (DE3) with a molecular weight ~75 kDa.