KLONING DAN EKSPRESI PROTEIN prM/E VIRUS DENGUE SEROTIPE 1 PADA *Escherichia coli*

INTISARI

Protein *precusor membrane*/*Envelope* (prM/E) Virus Dengue (DENV) dilaporkan merupakan antigen yang imunogenik dan dapat memunculkan respon imun yang tinggi dibandingkan dengan protein struktural maupun non-struktural yang lain, sehingga protein tersebut dapat digunakan sebagai kandidat vaksin. Penelitian ini bertujuan mengkloning gen dan mengekspresikan protein prM/E DENV dari isolat lokal. Sampel DENV dikonfirmasi serotipenya menggunakan *one-step multiplex* RT-PCR. Gen prM/E dari DENV-1 diamplifikasi menggunakan *two-step* RT-PCR. Kloning dan ekspresi protein prM/E dilakukan menggunakan vektor pGEMT *easy* dan pET32a yang ditransformasikan pada sel kompeten *E. coli* DH5α dan *E. coli* BL21 DE3. Bakteri *E. coli* BL21 DE3 yang positif membawa gen prM/E di induksi menggunakan IPTG 1 mM. Ekspresi protein prM/E dianalisis menggunakan SDS-PAGE, dan dipurifikasi menggunakan Ni-NTA agarose. Hasil penelitian menunjukkan gen prM/E sebesar 1983 bp berhasil di amplifikasi dan diperbanyak dalam *E. coli* DH5α. Protein prM/E yang berukuran sekitar 91-97 kDa dapat diekspresikan pada *E. coli* BL21 DE3, ditandai dengan hasil analisis SDS-PAGE yang menunjukkan adanya *band* diantara 85-120 kDa. Protein prM/E berhasil dipurifikasi menggunakan Ni-NTA agarose, meskipun hasilnya belum murni dan konsentrasinya relatif rendah. Berdasarkan hasil penelitian, disimpulkan bahwa gen prM/E Virus Dengue serotipe 1 isolat lokal dapat dikloning dan diekspresikan proteinnya pada *E. coli.*

Kata kunci: Virus Dengue, serotipe 1, prM/E, pET32a, *E. coli* BL21 DE3.

CLONING AND EXPRESSION OF prM/E PROTEIN OF DENGUE VIRUS SEROTYPE 1 IN *Escherichia coli*

ABSTRACT

precusor membrane/Envelope (prM/E) protein of Dengue Virus (DENV) has previously been reported as an immunogenic antigen and that it could trigger higher immune response compared to other structural and non structural proteins, therefore such protein can be used as a vaccine candidate. The objectives of this research are to clone genes and express proteins prM/E DENV from local isolate. DENV serotypes confirmed using one-step multiplex RT-PCR. prM/E gene was amplified using two-step RT-PCR. Cloning and expression of prM/E proteins were carried using pGEMT *easy* and pET32a vector, then were transformed into *E. coli* DH5α and *E. coli* BL21 DE3. *E. coli* BL21 DE3 which was positevely carried prM/E gene was being induced using 1 mM IPTG. Expression of prM/E protein were analyzed using SDS-PAGE and purified using Ni-NTA agarose. The result showed that prM/E gene of 1983 bp can be amplified and copied into *E. coli* DH5α. prM/E protein, which is about 91-97 kda, can be expressed on *E. coli* BL21 DE3, noted by SDS-PAGE analysis result which showed band on 85-120 kDa. prM/E protein was able to be purified using Ni-NTA agarose, although the result was not entirely pure and the concentration was relatively low. Based on this study, it can be concluded that prM/E gene of Dengue Virus serotype 1 from local isolate can be cloned and expressed in *Escherichia coli*.

Keyword: Dengue Virus, serotype 1, prM/E, pET32a, *E. coli* BL21 DE3.