Kloning gen rantai penyusun fab anti-NS1 virus dengue dari sel hibridoma 71E2 pada vektor pTA2 dalam escherichia coli TOP10 = Cloning of chain gene composing anti-NS1 fab dengue virus from hybridoma cell 71E2 on pTA2 vector in escherichia coli TOP10

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Abstrak

Infeksi dengue merupakan salah satu masalah utama kesehatan di dunia, termasuk di Indonesia. Angka kejadian demam berdarah dengue di Indonesia terus meningkat sejak tahun 1968 sampai tahun 2013. Akan tetapi, laju kematian atau case fatality ratio akibat demam berdarah dengue mengalami penurunan karena adanya deteksi infeksi dengue secara dini menggunakan antibodi monoklonal dan protein biomarka non-struktural 1 (NS1). Pengembangan antibodi monoklonal dengan teknologi hibridoma sebagai bahan baku kit diagnostik dengue terkendala masalah biaya produksi yang mahal dan waktu produksi yang relatif lama, sehingga dibutuhkan alternatif lain seperti pengembangan antibodi rekombinan. Penelitian ini dilakukan untuk memperbanyak gen rantai berat (heavy chain) dan rantai ringan (light chain) penyusun fragment antigen binding (Fab) rekombinan dari sel hibridoma lokal 71E2 yang telah diinduksi dengan virus dengue sehingga diharapkan mampu mensekreksikan antibodi anti-NS1. Gen heavy chain dan light chain diamplifikasi dengan menggunakan teknik polymerase chain reaction (PCR), kemudian divisualisasi dengan menggunakan elektroforesis gel agarosa. Produk PCR gen heavy chain menghasilkan pita berukuran 600 bp, sedangkan produk PCR gen light chain menghasilkan pita berukuran 350 bp. Plasmid rekombinan yang terdiri atas gen heavy chain/light chain dan vektor pTA2 ditransformasi ke dalam Escherichia coli TOP10 dengan menggunakan metode heat shock. Teknik PCR, digesti, dan sekuensing digunakan untuk mengkonfirmasi keberadaan gen target pada plasmid rekombinan. Hasil yang diperoleh menunjukkan bahwa gen heavy chain dan light chain yang berasal dari sel hibridoma 71E2 berhasil dikloning pada vektor pTA2 di dalam E. coli TOP10. Akan tetapi, diperlukan studi lebih lanjut untuk mengevaluasi kemampuan ekspresi Fab rekombinan yang tersusun atas gen heavy chain dan light chain hasil kloning sebagai material penting dalam pengembangan kit diagnostik dengue.
<hr><i>Dengue infection is one of major health problem which spread worldwide including in Indonesia. The dengue hemorrhagic fever (DHF) incidence in Indonesia increased rapidly from 1968 until 2013. However, the case fatality ratio decreased during the same period due to early detection of dengue infection by using monoclonal antibody and non-structural 1 (NS1) protein biomarker. The development of monoclonal antibody with hybridoma technology as raw material for dengue diagnostic kit was constrained by the expensive production costs and relatively long production time so that other alternatives are needed such as the development of recombinant antibody. This early study was conducted to clone heavy chain and light chain gene of recombinant fragment antigen binding (Fab) using local hybridoma cell 71E2 secreting monoclonal antibody anti-NS1 which was induced by dengue virus. The heavy chain and light chain gene of Fab were amplified by polymerase chain reaction (PCR) and then visualized by agarose gel electrophoresis. Heavy chain PCR product produces a band at 600 bp, while light chain PCR product produces a band at 350 bp. The recombinant plasmid that consists of the gene and pTA2 vector were transformed to Escherichia coli TOP10 using heat shock method. Polymerase chain reaction, digestion, and

sequencing method then used to confirm the gene insertion in the recombinant plasmid. The results showed that the heavy chain and light chain gene from hybridoma cell 71E2 were successfully cloned on the pTA2 vector in E. coli TOP10. However, further study should be conducted to evaluate the expression of the recombinant Fab from heavy chain and light chain gene as a valuable material in the development of dengue diagnostic kit.</i>