Kloning ekspresi, dan purifikasi protein NS1 virus dengue serotipe 3 isolat Jakarta = Cloning expression and purification NS1 protein dengue virus serotype 3 isolate Jakarta

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Abstrak

ABSTRAK
Gen NS1 merupakan gen penyandi protein NS1 (non-strukural 1) yang terdapat pada virus dengue. Protein NS1 diketahui memiliki potensi untuk dikembangkan sebagai bahan dasar kit diagnostik untuk penyakit demam berdarah dengue (DBD). Penelitian ini bertujuan untuk memperoleh protein rekombinan NS1 virus dengue untuk pengembangan kit diagnostik NS1. Penelitian ini meliputi proses kloning gen NS1 pada vektor ekspresi pYES2/CT, ekspresi pada Saccharomyces cerevisiae INVSc1 dan purifikasi protein rekombinan NS1 menggunakan HisPurTM Ni-NTA Magnetic Beads. Hasil penelitian menunjukkan sebanyak 485 koloni transforman hasil kloning ke dalam E. coli TOP10F? berhasil diseleksi pada medium yang mengandung 100 µg/ml. Analisis hasil PCR dan sekuensing menunjukkan bahwa gen NS1 yang berukuran 1056 pb berhasil terintegrasi ke dalam vektor ekspresi pYES2/CT. Analisis hasil SDS PAGE dan western blotting menunjukkan protein rekombinan NS1 berhasil diekspresikan pada Saccharomyces cerevisiae dengan ukuran sekitar 42?55 kDa. Analisis SDS PAGE untuk hasil purifikasi menunjukkan didapatkan protein yang terelusi dalam kondisi native dengan ukuran sekitar 42?55 kDa. Gen NS1 telah berhasil dikloning dan protein NS1 berhasil terekspresi serta terpurifikasi.
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ABSTRAK
NS1 gene is a gene encoding NS1 (non-structural 1) protein dengue virus. Dengue NS1 protein (non-structural 1) is known as an important biomarker for early diagnosis of dengue hemorrhagic fever (DHF) disease. The research objective is to obtain NS1 recombinant protein dengue virus serotype 3 for development kit diagnostic NS1. Stages of the research include cloning NS1 gene Into pYES2/CT expression vector, expression in Saccharomyces cerevisiae INVSc1, and purification NS1 recombinant protein with HisPurTM Ni-NTA Magnetic Beads. A total of 485 colony transformants were selected on medium with ampicilin 100 µg/ml as cloning results in E. coli TOP 10F?. PCR and sequencing analysis showed that NS1 gene was successfully fused to vector pYES2/CT and showed NS1 size is 1056 bp. SDS PAGE and western blotting analysis showed a band of NS1 recombinant protein as expression results. Molecular weight of NS1 protein was approximately 42--55 kDa. SDS PAGE analysis showed a band of NS1 recombinant protein purified in native condition with a molecular weight approximately 42--55 kDa. NS1 gene was successfully cloned, can be also expressed and purified as protein NS1.