Uji validitas hasil kloning, ekspresi, dan purifikasi protein rekombinan subunit ns3 dengue virus denv serotipe 3 asal Jakarta = Validity test of cloning result expression and purification of ns3 dengue virus denv serotype 3 subunit recombinant protein from Jakarta

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Abstrak

Protein NS3 pada Dengue Virus DENV Serotipe 3 DENV-3 adalah protein nonstruktural yang memiliki berat molekul 72 kDa dan bertanggung jawab dalam siklus replikasi virus dengue. Protein tersebut dapat dijadikan kandidat vaksin rekombinan subunit penyakit Demam Berdarah DBD . Penelitian ini bertujuan untuk validasi hasil kloning gen NS3 DENV-3 ke vektor pYES2/CT sebelumnya, ekspresi dan purifikasi protein rekombinan dari sel Saccharomyces cerevisiae. Uji validitas dengan metode PCR dan analisa sekuensing DNA menunjukkan bahwa gen NS3 DENV-3 pada klon 2 dan 11 memiliki validitas yang tinggi terinsersi pada plasmid pYES2CT >90. Hasil ekspresi transforman Saccharomyces cerevisiae pYES2/CT dengan metode SDS PAGE dan Western Blot menunjukkan adanya pita spesifik berukuran 72 kDA pada sampel 2A dan 8B. Hasil purifikasi sampel yang sudah terverifikasi ekspresinya sampel 2A dengan mekanisme elusi gradien menunjukkan adanya protein spesifik yang terelusi dengan menggunakan elution buffer yang mengandung imidazol konsentrasi 350 mM.

.....DENV 3 NS3 Protein is a non structural protein with molecular weight approximately around 72 kDA and responsible for replication cycle dengue virus. This protein could be a candidate for subunit recombinant vaccine of Dengue Haemorrhagic Fever DHF. The aims of this study were to validated the previous cloning result of DENV3 NS3 gene into pYES2 CT vector, expressed and purified the recombinant protein from Saccharomyces cerevisiae cells. The result of validity tests with PCR method and DNA sequencing showed NS3 gene in clone 2 and 11 had high validity were inserted on plasmid pYES2 CT 90. The result of the expression in transformant Saccharomyces cerevisiae pYES2 CT with SDS PAGE and Western Blot methods showed there was a specific band with size 72 kDa in clone 2A and 8B. The result of verified clone clone 2A with gradient elution mechanism showed there was a specific protein that was eluted by elution buffer which contained 350 mM imidazole.