Pengaruh berbagai konsentrasi isopropil-beta-D-thiogalactopyranoside (IPTG) terhadap ekspresi protein rekombinan jembrana superficial unit (JSU) pGEX-6P1

Ratih Cempaka, author

Abstrak

Protein Jembrana Superficial Unit (JSU) dapat dijadikan sebagai vaksin untuk pengobatan penyakit Jembrana. Protein JSU, yang dikode oleh gen env, disisipkan ke dalam plasmid pGEX-6P1 dan diekspresikan melalui Escherichia coli strain BL21 sebagai inangnya. Tujuan penelitian adalah untuk meneliti berbagai pengaruh konsentrasi IPTG terhadap ekspresi protein rekombinan JSU pGEX-6P1. Sel E. coli (pembawa konstruk pGEX-6P1) ditumbuhkan pada medium Luria Betani (LB) cair 50 ml dan diinkubasi pada shaker incubator hingga mencapai kepadatan sel (OD) OD600 0,6. Induksi Isopropyl--Dthiogalactopyranoside (IPTG) selama 1 jam dengan tiga konsentrasi perlakuan, yaitu 100 M, 150 M, dan 200 M. Sel dipecah dengan dua metode, yaitu Freeze and thaw dan sonikasi kemudian pelet hasil pemecahan sel dikoleksi sebagai inclusion body. Solubilisasi protein dilakukan dengan menambahkan solubilize buffer pada pelet kemudian dilution buffer untuk tahap refolding. Protein dimurnikan melalui Gluthatione Sepharose 4B dengan metode batch capture. Hasil analisis SDS-PAGE menunjukkan ukuran protein JSU pGEX-6P1 yang tepat, yaitu ± 60 kDa pada setiap perlakuan (konsentrasi) IPTG. Pita pada induksi IPTG 100 M terlihat lebih tebal dibandingkan dengan pita pada induksi 150 M dan 200 M. Hasil penelitian disimpulkan bahwa induksi IPTG 100 M menghasilkan protein rekombinan JSU pGEX-6P1 yang optimal.

.....Jembrana Superficial Unit (JSU) protein can be used as a vaccine material for controlling Jembrana disease. JSU protein that encoded by the env gene was inserted into the plasmid pGEX-6P1 and expressed through the Escherichia coli strain of BL21 as a host. The aim of this study was to determine the effect of IPTG concentrations against the expression of JSU pGEX-6P1 recombinant protein. E. coli cells (pGEX-6P1 constructs carrier) were grown in 50 ml Luria Bertani (LB) liquid medium and was incubated on a shaker incubator until it reaches the cell density (OD) OD600 0.6. Induction of Isopropyl--Dthiogalactopyranoside (IPTG) for 1 hour with three concentrations of the treatment, there are 100 M, 150 M, and 200 M. The cells was disrupted by two methods of cell lysis, Freeze and thaw and sonication, then the pellet was collected as inclusion body. Protein is solubilized by adding a buffer into the pellet and using dilution buffer for refolding step. Proteins purified using Gluthatione Sepharose 4B by batch capture method. The analysis of SDS-PAGE was shown exactly the protein size of JSU pGEX-6P1 ± 60 kDa for each treatment (concentration) IPTG. Band at 100 M IPTG induction seems thicker than the band on the induction of 150 M and 200 M. The study was concluded that 100 M IPTG induction produces an optimal of JSU pGEX-6P1 recombinant protein.