Strategi Ekspresi dan Purifikasi Protein Rekombinan Tat-Eli HIV-1 menggunakan Escherichia coli = Strategy of Expression and Purification Tat-Eli HIV-1 recombinant protein using Escherchia coli

Chairunisa Fadhilah, author

Abstrak

Protein Transaktivator transkripsi (Tat) adalah protein regulator HIV-1 berfungsi sebagai aktivator transkripsi genom HIV-1. Varian protein Tat-Eli adalah aktivator transkripsi paling kuat daripada varian lain melalui induksi promotor LTR HVI-1. Kemampuan tersebut digunakan sebagai kontrol positif dalam pengembangan uji infeksitivitas HIV-1 berbasis gene reporter eGFP diregulasi LTR HIV-1. Pengembangan uji infeksivitas tersebut menawarkan waktu deteksi infeksi lebih singkat daripada uji p24 pada uji fenotipik. Penelitian ini bertujuan untuk mengekspresikan protein rekombinan Tat-Eli di sistem ekpresi prokariot dan mempurifikasinya sehingga dapat dijadikan kontrol positif penginduksi promotor. Pada penelitian ini dilakukan pengklonaan gen sintetik Tat-Eli ke vektor pQE80L. Protein rekombinan Tat-Eli dipurifikasi menggunakan Ni-NTA. Pengklonaan ulang gen reporter eGFP disisipkan setelah promotor LTR HIV-1. Aktivitas protein rekombinan Tat-Eli terhadap ekspresi eGFP di sel mamalia dinilai berdasarkan persentase sel pengekspresi eGFP dan intensitas cahaya eGFP.Konstruksi plasmid rekombinan membawa gen Tat-Eli, pQETat, berhasil dibuat, diekspresikan dan dipurifikasi kondisi native. Plasmid pengekspresi eGFP dengan promoter HIV-1, pLTReGFP berhasil dikonstruksi. Penambahan Tat-Eli rekombinan pada sel mamalia yang ditransfeksi pLTReGFP menunjukkan perbedaan intensitas cahaya eGFP yang bermakna dan paling tinggi dari semua perlakuan. Protein rekombinan Tat-Eli dapat diekspresikan dan dipurifikasi secara optimal dari E.coli. Penambahan protein Tat-Eli pada sel yang ditransfeksi pLTReGFP meningkatkan intensistas cahaya eGFP.

.....Transcriptional Transactivator Protein (Tat) is an HIV-1 regulatory protein functioning as an activator of HIV-1 genome transcription. The Tat-Eli protein variant was the most potent transcriptional activator than other variants through the induction of the HVI-1 LTR promoter. This ability was used as a positive control in the development of an HIV-1 infection test based on the eGFP reporter gene regulated by LTR HIV-1. The development of the infectiousness test offers a shorter infection detection time than the p24 test in the phenotypic test. This study aims to express Tat-Eli recombinant protein in the prokaryotic expression system and to purify it so that it can be used as a positive control inducer of the promoter. In this study, synthetic Tat-Eli gene was cloned into the pQE80L vector. Tat-Eli recombinant protein was purified using Ni-NTA. Recloning of the eGFP reporter gene was inserted after the HIV-1 LTR promoter. The activity of Tat-Eli recombinant protein on eGFP expression in mammalian cells was assessed based on the percentage of eGFP-expressing cells and eGFP light intensity. The recombinant plasmid construction carrying the Tat-Eli gene, pQETat was successfully generated, expressed and purified in native conditions. An eGFP-expressing plasmid with HIV-1 promoter, pLTReGFP was successfully constructed. The addition of recombinant Tat-Eli to mammalian cells transfected with pLTReGFP showed a significant difference in eGFP light intensity and was the highest of all treatments. Tat-Eli recombinant protein can be optimally expressed and purified from E. coli. The addition of Tat-Eli protein in pLTReGFP-transfected cells increased eGFP light intensity.