Expression of HIV-1 Recombinant Protein gp-41 in plasmid pQE80L of Escherichia coli for Development of HIV-1 Diagnostic System = Ekspresi Protein Rekombinan HIV-1 gp-41 menggunakan Plasmid pQE80L pada Escherichia coli untuk pengembangan sistem diagnostik HIV-1

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Abstrak

Latar Belakang: gp41 adalah transmembran glikoprotein yang memiliki peran penting dalam fusi dan masuknya Human Immunodeficiency Virus-1 (HIV-1). Keterlibatan protein transmembrane gp41 sangatlah krusial di seluruh fase awal penularan mukosa HIV-1; maka keberadaan protein ini penting untuk skrining dan diagnosis HIV-1. Protein transmembran gp41 dapat dimanfaatkan sebagai alat diagnostik HIV-1 melalui uji deteksi antibodi yang meliputi, Rapid Diagnostic Test, ELISA, dan Western Blot (WB). Namun, meskipun terdaftar sebagai salah satu protein HIV-1 yang memiliki banyak manfaat, studi mengenai ekspresi dan optimalisasi protein transmembran gp41 masih kurang diteliti. Oleh karena itu, penelitian ini bertujuan untuk memperoleh pengetahuan mengenai kondisi optimal untuk mengekspresikan protein transmembran gp41 pada Escherichia coli (E. coli) PQE80L untuk pengembangan uji diagnostik HIV-1.
Metode: Penelitian ini menggunakan bagian imunodominan dari protein transmembrane gp41 (gp41-IDR). Protein gp41-IDR kemudian diekspresikan dalam sistem ekspresi E. coli dan dioptimalkan pada berbagai variabel, yaitu media kultur, konsentrasi penginduksi dan waktu induksi. Hasil yang diperoleh dari SDS-PAGE 20% didokumentasikan oleh ImageQuant Las 4000, sedangkan kuantisasi dan analisa protein gp41-IDR dilakukan di Image Lab 6.1. Software for Mac.
Hasil: Hasil penelitian menunjukkan bahwa protein gp41-IDR lebih maksimal jika diekspresikan pada medium Terrific Broth (TB) dibandingkan dengan dua media kaya nutrisi lainnya, yaitu Luria Bertani (LB) dan 2x Yeast Extract-Tryptone (2x YT). Di antara lima konsentrasi Isopropil-beta-D-thiogalactopyranoside (IPTG) yang diuji, induksi oleh 1 mM IPTG menunjukkan hasil protein tertinggi jika dibandingkan dengan konsentrasi IPTG lainnya. Apabila dibandingkan dengan protein yang diinduksi selama 6 jam dan semalam, induksi protein gp41-IDR rekombinan selama 3 jam menunjukkan hasil protein tertinggi.
Kesimpulan: Protein rekombinan gp41-IDR HIV-1 berhasil diekspresikan pada Escherichia coli (E. coli), dengan PQE80L sebagai vektor ekspresi. Hasil dari penelitian ini menunjukkan bahwa protein rekombinan gp41-IDR dari HIV-1 Subtipe CFR01_AE diekspresikan secara optimal pada medium Terrific Broth (TB), dengan 1 mM IPTG selama 3 jam.
.....Background: gp41 is a viral transmembrane glycoprotein that plays a significant role in the fusion and entry of Human Immunodeficiency Virus-1 (HIV-1). The involvement of gp41 transmembrane protein is pivotal throughout the early phases of HIV-1 mucosal transmission; hence the presence of this protein is important for HIV-1 screening and diagnosis. gp41 transmembrane protein can be utilized as an HIV-1 diagnostic tool through antibody detection tests, namely Rapid Diagnostic Test, ELISA, and Western Blot (WB). However, despite being listed as one of the most valuable HIV-1 proteins, research regarding the expression and optimization of gp41 transmembrane protein is likely underreported. Therefore, this research aims to obtain knowledge regarding the optimal condition to express gp41 transmembrane protein in

Escherichia coli (E. coli) PQE80L for the development of HIV-1 diagnostic test.
Methods: The immunodominant region of gp41 transmembrane protein (gp41-IDR) was used in this study. gp41-IDR protein was expressed in E. coli expression system and optimized under varying variables, namely culture medium, inducer concentration and induction time. The results obtained from SDS-PAGE 20% were documented by ImageQuant Las 4000, while quantitation and analysis of the gp41-IDR protein was done in Image Lab 6.1. Software for Mac.
Results: The results indicated that the gp41-IDR protein yield was maximized when expressed in Terrific Broth (TB) Medium as compared to other two nutrient-rich media, namely Luria Bertani (LB) and 2x Yeast Extract-Tryptone(2x YT). Among the five Isopropyl-beta-D-thiogalactopyranoside (IPTG) concentrations tested, induction by 1 mM of IPTG showed the highest protein yield when compared to the other IPTG concentrations. In comparison to those induced for 6 hours and overnight, induction of recombinant gp41-IDR protein for 3 hours offered the highest protein yields.
Conclusion: To conclude, recombinant gp41-IDR HIV-1 protein was successfully expressed in the <i>Escherichia coli (E. coli) host system, with PQE80L as expression vector. Findings from this study indicate that gp41-IDR recombinant protein from HIV-1 Subtype CFR01_AE is optimally expressed in Terrific Broth (TB) Medium, with 1 mM of IPTG for 3 hours.