Produksi dan purifikasi protein human granulocyte colony stimulating factor dari pichia pastoris rekombinan = Production and purification protein of human granulocyte colony stimulating factor from recombinant pichia pastoris

Sembiring, Enny Rimita, author

Abstrak

Granulocyte Colony Stimulating Factor (G-CSF) merupakan faktor pertumbuhan hematopoetik yang berfungsi merangsang proliferasi dan diferensiasi neutrofil. Protein G-CSF rekombinan yang dikembangkan dan diproduksi menggunakan sel inang Escherichia coli dan Chinese Hamster Ovary (CHO) masih memiliki kelemahan, sehingga pada penelitian ini dikembangkan suatu produk biosimilar G-CSF rekombinan menggunakan sel inang Pichia pastoris. Fokus penelitian ini adalah memproduksi dan mempurifikasi protein G-CSF rekombinan. Produksi protein rekombinan dilakukan dengan menginduksi kultur menggunakan metanol konsentrasi 0,5% tiap 12 jam dan dilakukan sampling terhadap kultur pada jam ke-0, 12, 24, 36 dan 48. Hasil analisis western blot menunjukkan adanya peningkatan produksi protein rekombinan tiap 12 jam. Protein G-CSF rekombinan dipresipitasi menggunakan amonium sulfat konsentrasi 80%, kemudian didialisis. Konsentrasi protein total diukur dengan spektrofotometer menggunakan metoda Bicinchoninic Acid (BCA). Hasil pengukuran menunjukkan konsentrasi protein total tertinggi adalah sampel protein yang dipresipitasi dengan 80% amonium sulfat. Selanjutnya, purifikasi dilakukan menggunakan teknik kromatografi afinitas dengan resin Ni-NTA. Hasil analisis SDS PAGE menunjukkan protein GCSF rekombinan berukuran 18,5 kDa dan dengan analisis slot blot terdeteksi berwarna ungu.
<hr>Granulocyte Colony Stimulating Factor (G-CSF) is a hematopoietic growth factor that acts to stimulate neutrophilic proliferation and differentiation. Recombinant protein G-CSF developed and produced using cellular host Escherichia coli and Chinese hamster ovary (CHO) still has a weakness, so that in this study we developed a bio similar product of recombinant G-CSF using cellular host Pichia pastoris. The aim of this research was to produce and purify recombinant protein G-CSF. Production of recombinant protein was done by inducing culture with methanol 0.5% every 12 hours and sampling was carried out at 0, 12, 24, 36 and 48 hours. The results of western blot analysis showed an increase the production of recombinant protein every 12 hours. Recombinant protein G-CSF was precipitated using ammonium sulfate 80% of concentration, and then dialyzed. Concentration of total protein was measured by a spectrophotometer using the Bicinchoninic Acid (BCA) method. The measurement results showed the highest concentrations of total protein was present in samples that precipitated with 80% ammonium sulfate. Furthermore, purification performed using affinity chromatography techniques with Ni-NTA resin. The results of SDS PAGE analysis showed the recombinant protein G-CSF sized 18.5 kDa and with a slot blot analysis detected a purple color.