Metode solubilisasi dan refolding protein rekombinan hvdac3 human voltage dependent anion channel isoform 3 tidak larut dari badan inklusi escherichia coli strain bl21 startm de3 = Solubilization and refolding method for insoluble recombinant protein hvdac3 human voltage dependent anion channel isoform 3 from inclusion bodies escherichia coli strain bl21 startm de3

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Abstrak

Penelitian mengenai metode solubilisasi dan refolding protein rekombinan hVDAC3 ekson 5?8 dari badan inklusi telah dilakukan. Penelitian bertujuan menganalisis dan membuktikan hubungan antara perlakuan deterjen LDAO dan lama waktu inkubasi terhadap perolehan konsentrasi protein rekombinan hasil solubilisasi dan refolding. Solubilisasi dilakukan dengan penambahan 6 M guanidin HCl sedangkan refolding dilakukan dengan perlakuan berbagai konsentrasi deterjen LDAO sebesar 0.5%, 1.0%, 1.5%, 2.0%, dan 2.5% serta perlakuan berbagai waktu inkubasi selama 0 jam, 24 jam, 48 jam, 72 jam, 96 jam, 120 jam, 144 jam, dan 168 jam. Setiap perlakuan dilakukan untuk mempertahankan protein rekombinan hVDAC3 dalam kondisi terlarut tanpa kembali membentuk protein agregat. Protein rekombinan hVADC3 tidak larut dalam badan inklusi diperoleh dari kombinasi sistem ekspresi antara plasmid pET100/D-TOPO dan Escherichia coli strain BL21 StarTM (DE3) dengan induksi ekspresi 1 mM IPTG.

Pilot project dilakukan untuk mengukur konsentrasi protein relatif menggunakan OD280 dan konsentrasi protein total menggunakan metode Bradford sebagai indikator perolehan protein terlarut hasil solubilisasi dan refolding. Perolehan protein rekombinan hVDAC3 hasil solubilisasi dan refolding dikonfirmasi secara kualitatif dengan metode western blotting dan secara kuantitatif dengan metode ELISA. Perlakuan deterjen LDAO terhadap perolehan konsentrasi protein total berbeda signifikan ($p < 0.05$), memiliki korelasi yang signifikan ($p < 0.05$), dengan nilai koefisien korelasi (R2) linear sebesar 0.884 dan arah korelasi bernilai positif. Dengan demikian perlakuan deterjen LDAO berpengaruh sangat kuat terhadap konsentrasi protein total. Semakin tinggi konsentrasi deterjen LDAO yang diberikan maka semakin tinggi pula konsentrasi protein total yang diperoleh. Perlakuan waktu inkubasi terhadap perolehan konsentrasi protein total tidak berbeda signifikan ($p > 0.05$), tidak memiliki korelasi yang signifikan ($p > 0.05$), dengan nilai koefisien korelasi (R2) linear sebesar 0.003 dan arah korelasi tidak terdefinisi. Dengan demikian perlakuan waktu inkubasi tidak berpengaruh terhadap konsentrasi protein total. Semakin lama waktu inkubasi yang dilakukan tidak memengaruhi perolehan konsentrasi protein total. Analisis kualitatif menunjukkan bahwa ukuran protein rekombinan hVDAC3 sebesar 25 kDa sedangkan analisis kuantitatif pada perlakuan deterjen LDAO 0.5% dan 2.5% dengan waktu inkubasi 24 jam berurutan sebesar 36.951 ± 5.679 dan 53.197 ± 23.694 µg/ml volume kultur.

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Research about method for solubilization and refolding insoluble recombinant protein hVDAC3 exon 5?8 from inclusion bodies has been done. The research aims to analyze and prove the relationship between LDAO detergent treatment and incubation time with protein concentration after solubilization and refolding

process. Solubilization is carried out with the addition of 6 M Guanidine HCl whereas refolding with various concentrations of LDAO (0.5%, 1.0%, 1.5%, 2.0%, and 2.5%) and various time incubation (0, 24, 48, 72, 96, 120, 144, and 168 hours). Each treatment was done to maintain recombinant protein hVDAC3 in soluble state without reforming protein aggregates. Insoluble recombinant protein hVDAC3 exon 5?8 in inclusion bodies obtained from a combination plasmid pET100/D-TOPO and Escherichia coli strain BL21 StarTM (DE3) expression system with 1 mM IPTG for induction.

Pilot project to measure the relative protein concentration using OD280 and total protein concentration using Bradford method as an indicator of protein amount that remained in solution after refolding. The acquisition of recombinant protein hVDAC3 after solibilization and refolding process confirmed qualitatively by western blotting and quantitatively by ELISA method. LDAO detergent treatment for total protein concentration is significant different ($p < 0.05$), had a significant correlation ($p < 0.05$), with a value of correlation coefficient (R2) 0.884 and positive direction of linear correlation. Thus the LDAO detergent treatment very strong influence on the total protein concentration. The higher concentration of LDAO detergent given the higher total protein concentrations were obtained. Incubation time treatment for total protein concentration did not differ significantly ($p > 0.05$), no significant correllation ($p > 0.05$), with a value of correlation coefficient (R2) 0.003 and a linear correlation direction is undefined. Thus incubation time treatment did not effect on total protein concentration. The longer incubation time do not effect the acquisition of the total protein concentration. Qualitative analysis showed the hVDAC3 recombinant protein was detected at 25 kDa while quantity of hVDAC3 recombinant protein for 0.5% and 2.5% LDAO concentration treatment with 24 hours incubation time consecutively 36.951 ± 5.679 and 53.197 ± 23.694 µg/ml culture volume.