عنوان مقاله:

A Convenient Method for Solubilization and Refolding Recombinant Proteins: An Experience from Recombinant Mouse TGF-β۱

محل انتشار:

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نویسندگان:

Fahimeh Maleki - *Immuno-Biochemistry lab, Immunology Research Center, Buali Research Institute, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*

Kazem Mashayekhi - *Immuno-Biochemistry lab, Immunology Research Center, Buali Research Institute, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*

Seyedeh Elham Badiee Kheirabadi - *Immuno-Biochemistry lab, Immunology Research Center, Buali Research Institute, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*

Mohammad Javad Mousavi - *Immunology Department, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran*

Mojtaba Sankian - *Immuno-Biochemistry lab, Immunology Research Center, Buali Research Institute, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*

خلاصه مقاله:

Background: The production of recombinant proteins in Escherichia coli is one of the most valuable achievements in biotechnology, with many therapeutic and diagnostic applications; however, the aggregation and misfolding of proteins that result in the formation of insoluble inclusion bodies is a disruptive factor in this process. Various solubilization and refolding methods can be used to improve recombinant protein conformation. In this study, we applied a dilution method with a refolding buffer to produce a native form of soluble immature mouse TGF-β۱. Materials and Methods: The TGF-β۱ cDNA which encodes the protein without the signal peptide, was cloned into the pET۲۱-b (+) vector. The target protein was expressed by the transformation of E. coli BL۲۱ cells with the plasmid. The resulting inclusion bodies were solubilized and then diluted in the refolding buffer to make a protein with native structure. Results: Following PCR of the recombinant plasmid with T۷ primers, electrophoresis and sequencing of the amplified product indicated ۱۰۰% identity of the target sequence with the murine TGF-β۱ gene. Finally, the protein solubility and immuno-reactivity were confirmed a ۴۴ kDa protein which conducted with the anti-TGF-β۱-specific polyclonal antibody on a western blot. Conclusion: Our dilution method and refolding buffer effectively converted aggregated immature mouse TGF-β۱ to a soluble and immuno-reactive form.

کلمات کلیدی:

Inclusion Bodies, Mouse TGF-β۱, protein expression, refolding protein