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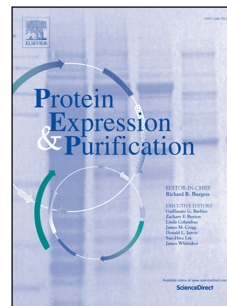

Expression and purification of the human epidermal growth factor receptor extracellular domain

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Expression and purification of the human epidermal growth factor receptor extracellular domain

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Abstract

In the fields of drug discovery and protein science, small quantities of proteins are always needed to investigate or validate protein–protein (or protein–small molecule) interactions. Traditional transient or stable expression method to obtain recombinant proteins in eukaryotic systems can be laborious and time-consuming, especially when multiple protein variants are required. Here, we present a fast and convenient method for obtaining small quantities of recombinant human epidermal growth factor receptor (rhEGFR) ectodomain protein, which could be efficiently extended to the expression of other eukaryotic proteins. Human *EGFR* ectodomain gene was inserted into the plasmid pBMN-GFP and recombinant plasmid was transfected into HEK 293T cells. In the presence of hygromycin, cells with the integrated human *EGFR* ectodomain gene were selected and proliferated. rhEGFR ectodomain in cell culture supernatant was purified using serial connected diethylaminoethyl Sepharose column and Ni-NTA Sepharose column. Purity of the final purified rhEGFR ectodomain was over 95% according to SDS-PAGE analysis. Moreover, the purified target protein was biological active via measuring the affinity between the rhEGFR ectodomain and rhEGF. Our method could greatly facilitate research in the areas of protein science, protein structural biology, and drug discovery.

Keywords: Epidermal growth factor receptor, Mammalian proteins, Recombinant protein

1. Introduction

In classical phenotypic drug discovery, when a compound manifests the desired function in cell- or function-based assays, the exact biological target must subsequently be identified [1, 2]. Once a potential target protein has been proposed, it will be expressed, purified, and used for further validation assays [3]. A variety of analytical techniques, such as surface plasmon resonance [4, 5], microscale thermophoresis [6], X-ray crystallography [7, 8], and dual-polarization interferometry [9], can be used to validate the interaction between a compound and potential target protein, which typically involves the quantification of reaction affinities and kinetic properties or identification of the binding site(s) and conformational changes [10].

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