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Expression and purification of constitutively active S6K1 using dual Bac-to-Bac protein expression system

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Aim. Since p70S6K1 plays a key role in protein synthesis and cell cycle regulation, it has been implicated in a number of human diseases, such as obesity, diabetes, and cancer. Increased activation of p70S6K1 has been reported in a variety of cancer types, so now it is being investigated as a potential therapeutic target. The purpose of this work was to create a novel more efficient approach of expression and purification of p70S6K1 constitutively active form by using Dual Bac-to-Bac protein expressing system. **Methods.** For the production of T-loop phosphorylated p70S6K1 protein we have used an insect Bac-to-Bac baculovirus expression system with pFastBac™ Dual vector, which possesses two multiple cloning sites controlled by polyhedrin promoter (PH) and p10 promoter, allowing the simultaneous expression of two heterologous genes. Therefore, we first constructed the pFastBac™ Dual plasmid loaded with 6His-p70S6K1(ΔAID)-T389D under the control of PH promotor together with GST-PDPK1(ΔPH) controlled by p10. For the protein production Sf9 cells were infected with recombinant bacmid DNA.The purification of p70S6K1 was carried out using Ni-NTA affinity chromatography and subsequent dialysis. The quality and concentration of obtained protein was tested using SDS-PAGE under reducing conditions. To detect the activity of p70S6K1protein, *in vitro* kinase assay against kinase's substrate — ribosomal protein S6, was used with following western blotting (WB). WBagainst phosphorylated by PDK1 sites of p70S6K1 was carried out to confirm the specificity and activity of the obtained recombinant kinase. **Results.** Here we demonstrate the novel approach of obtaining highly active recombinant p70S6K1 (His-actS6K1) using dual baculovirus vector co-expressing 6His-p70S6K1(ΔAID)-T389D together with GST-PDPK1(ΔPH), assuring the synthesis of both proteins by the infected cell. For this study the initial p70S6K1 was modified by addition of 6His-tag at N-terminal regulatory domain, deletion of C-terminal domain containing autoinhibitory motif (AID), and with mutation of T389 site to D389 to mimic the phosphorylation by mTORC1. This results in guaranteed and more efficient phosphorylation and following activation of p70S6K1, which was confirmed using *in vitro* kinase activity assay and WB. **Conclusions.** The current work was focused on the design of a new highly efficient system for the expression and purification of the recombinant His-actS6K1, using pFastBac™ Dual vector co-expressing recombinant GST-PDK1. Recent studies have shown that some cancer types demonstrate the enhanced expression and/or activation of p70S6K, hinting that it may serve as a biomarker for monitoring disease progression. With that in mind, our approach of large-scale expression of His-actS6K1 may offer a rapid, low-cost, and high yield method for producing constitutively active recombinant p70S6K1 and facilitate the development of its potential therapeutic regulators.

Keywords: protein expression, protein phosphorylation, S6K1, Baculovirus expression system, kinase activity.