Cell-Free Synthesis of a High Molecular Weight Protein Using *ExiProgen*™ Automated Protein Synthesis System

Yousang Cho[1], Junho Jung[1], Nayoung Lee[1], and Jiwon Han[1*]
[1]Molecular Science laboratory, Bioneer Corporation, 8-11Munpyeongseoro, Daedeok-gu, Daejeon 306-220, Korea
[*]Email: exiprogen@bioneer.co.kr, exiprogen-support@bioneer.co.kr

Introduction

Cell-free protein synthesis (*in vitro* protein transcription and translation) is a common method to synthesize a desired protein in a rapid and efficient manner. It is accomplished by adding template DNA (containing the coding region of the protein of interest) into a single tube which contains cell extracts and other ingredients essential for protein synthesis. Because this method does not require a separate cell line selection step, it is able to yield diverse types of protein in a very short period of time when compared to *in vivo* protein expression. Hence, cell-free protein synthesis has potential advantages in the high-throughput synthesis of proteins (1-3).

ExiProgen protein synthesis system applies automation to *in vitro* protein expression and magnetic bead-based His-Tag affinity purification and yields highly-pure proteins. *In vitro* protein synthesis using *ExiProgen* (Bioneer, Cat. No. A-5041) has a simple workflow; template DNA preparation, loading template DNA into ExiProgen EC1 protein synthesis kit (Bioneer, Cat. No. K-7300), loading the kit components onto the deck of *ExiProgen*, and starting the system. *ExiProgen* EC1 protein synthesis kit contains optimized *E. coli* extract which has T7 RNA polymerase and ribosomes, all other required components such as amino acids and an energy source for effective and efficient *in vitro* transcription and translation. The kit also contains Ni-NTA magnetic beads for purification of expressed his-tagged proteins. *ExiProgen* EC1 protein synthesis kit has 16 reaction wells so that up to 16 different kinds of highly pure proteins can be obtained at the same time within 6 hours of adding template DNA which can be in the form of a plasmid or linear PCR products.

In this study, we synthesized *Bacillus megaterium* BM3 protein *in vitro* using *ExiProgen* protein synthesis system to demonstrate *ExiProgen*'s capability to automatically express and purify proteins as large as 117 kDa.

Methods and results

To synthesize BM3 protein (MW 117 kDa), 10 ug of BM3 expression vector for *in vitro* expression (a kind gift from Dr. Dong-Myung Kim at Chungnam National University) was added into a reaction well of the protein expression cartridge (cartridge 2) of *ExiProgen* EC1 Protein Synthesis Kit. The kit contents, including protein purification cartridge (cartridge 1), *E.coli* cell extract, elution tubes, and filter tips as well as cartridge 2, were then placed in the correct position on the deck of the *ExiProgen*. Next, *ExiProgen* was run after selecting the protocol number 902 as described in the kit manual. The run was finished in less than 6 hours and 250 µl of purified protein samples were collected in the elution tubes. To check the synthesis of the target proteins, the expression samples from J section of cartridge 2 and purification samples in the elution tubes were run in the SDS-PAGE gel. Samples were prepared for SDS-PAGE gel analysis as described in the manual. The SDS-PAGE result showed that BM3 was expressed and purified with *ExiProgen* (Figure 1). The amount of synthesized/purified BM3 was determined to be about 60 ug per reaction.

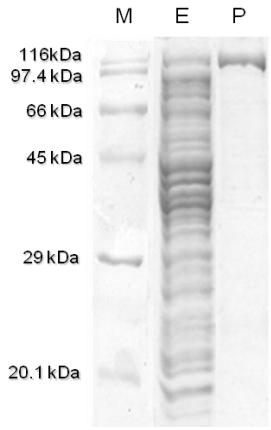

Figure 1. Purification and expression of BM3. *ExiProgen* is able to synthesize proteins as large as 120 kDa. Lane M: *AccuLadder*™ Protein Size Marker (Broad), Lane E: Unpurified expression sample, Lane P: Purified sample

In conclusion, we successfully expressed and purified BM3 with *ExiProgen*, indicating that *ExiProgen* is able to synthesize proteins as large as 117 kDa. Since it is automated, easy-to-use, and provides rapid protein synthesis, *ExiProgen* has the potential applications several research fields including identification of protein function, protein-protein interaction study and protein structure labs as well as enzyme engineering and in Biofuel research labs. *ExiProgen* is proving to be a valuable tool for protein scientists.

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References

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