CellFree Sciences

The natural power of wheat driving science

High Performance Cell-Free Wheat Germ Protein Expression System

Application Note

Application to protein complex synthesis

There are more than 36.7 million people in the world infected with HIV, the pathogenic virus that causes AIDS, with nearly 18,000 newly infected cases being reported each year (as of the end of 2016). Although the establishment of cocktail therapy has improved the survival prognosis for infected patients, resistance to existing drugs has emerged, and various studies are currently underway to develop new treatments. The expression of the intracellular signaling regulator SOCS1 is thought to be induced in HIV-infected cells. SOCS1 forms a ubiquitin ligase complex around the nucleus and stabilizes intercellular transport of the Gag protein, an essential viral particle component. In this study, we attempted to synthesize a ubiquitin ligase complex (E3 complex) composed of four types of proteins, including SOCS1, by taking advantage of the coexpression abilities of the wheat germ cell-free protein expression system.

Method

Template plasmid construction

SOCS1 forms a complex with four other proteins: Cullin2 (Cul-2), Rbx1, Elongin B (B), and Elongin C (C) (Figure 1).

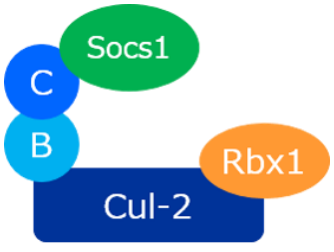

Figure 1: Diagram of SOCS1 protein complex

Each gene was cloned into an expression vector optimized for our wheat cell-free expression system to create a template for the expression. In the pre-test, individual synthesis of Cullin2 produced the lowest yield. Therefore, we added a GST tag to its N-terminus. A protease was then used to remove the GST tag after the synthesis through pull-down purification.

Protein synthesis and purification

After mRNA pieces were transcribed from the constructed templates, they were distributed and coexpressed in dialysis cups together with the wheat germ extract WEPRO7240G and translation buffer SUB-AMIX® SGC.

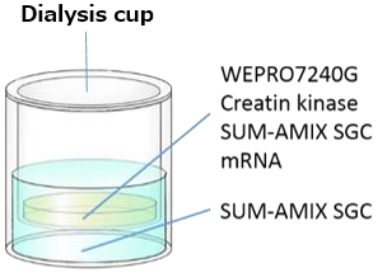

Figure 2: Translation-response using a dialysis cup

CellFree Sciences Co., Ltd., Yokohama Bio Industry Center, 1-6 Suehiro-cho, Tsurumi-ku, Yokohama, Kanagawa 230-0045, Japan
Tel: +81-(0)45-345-2625 Fax: +81-(0)45-345-2626 Web: http://cfsciences.com/eg/index.html

After synthesis, the GST tag was excised by protease treatment (PreScission Protease) and the E3 complex was purified through affinity with GST. The purified E3 complex was concentrated and the buffer was exchanged using Microcon.

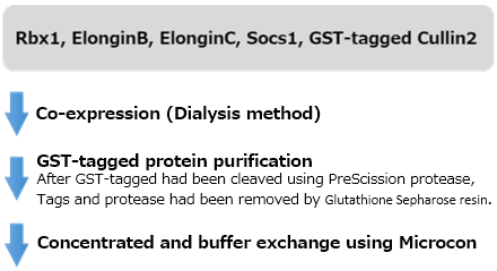

Figure 3: Flow of operations

Protein synthesis confirmation

SDS-PAGE and CBB staining were used to confirm the synthesis and purification of the complex.

Results

Synthesis and purification of the E3 complex

Figure 4 shows the results of SDS-PAGE and CBB staining, confirming the presence of the purified yield of the E3 complex.

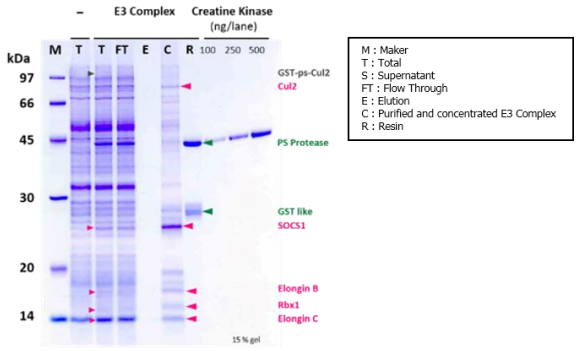

Figure 4: Synthesis and purification results of the E3 complex

References

1. Nishi et al., Requirement for microtubule integrity in the SOCS1-mediated intracellular dynamics of HIV-1 Gag. FEBS Letters, 2009. 583: p.1243-1250