CellFree Sciences

The natural power of wheat driving science

High Performance Cell-Free Wheat Germ Protein Expression System

Application Note

New BD reaction format for expression of membrane proteins and direct preparation of asolectin-based proteoliposomes

Abstract

Membrane proteins are the most important drug targets we have today. However, studies on those proteins remain difficult as the preparation of recombinant membrane proteins offers many challenges. These challenges can be addressed by using the ProteoLiposome BD Expression Kit and new BD ("bilayer-dialysis") reaction format for the direct preparation of proteoliposomes in a simple cell-free protein expression reaction in the presence of added liposomes made from asolectin.

Introduction

Nearly a third of all eukaryotic genes encode membrane proteins [1], many functioning as essential drug targets [2]. Only G protein-coupled receptors (GPCRs) are targeted today by about 40-50% of all marketed drugs [3]. Despite their importance, studying membrane proteins remains slow, largely because their extraction from biological samples and their expression as recombinant proteins is complicated [4]. The overexpression of membrane proteins in cell-based expression systems is limited by toxicity to the cell host. Furthermore, membrane proteins tend to form insoluble aggregates when they are not embedded into a lipid bilayer. However, for functional protein analysis or use as antigens recombinant proteins are required.

For long proteoliposomes have been used to study membrane proteins, where the proteins are integrated into the lipid bilayer of liposomes (artificially-prepared spherical lipid vesicles) [5]. Integration into liposomes supports proper folding of transmembrane domains and hence makes it more likely to obtain also active proteins. Early studies on membrane biogenesis and signaling peptides indicated, that cell-free protein expression systems in combination with lipids or membrane vesicles isolated from biological samples can be used to express and analyze membrane proteins [6, 7]. Several membrane proteins were studied in this way including a functional lactose permease that could be prepared combining inverted membrane vesicles from *E. coli* with an *E. coli* cell-free expression system [8]. When the inverted membrane vesicles were added cotranslationally to the protein expression reaction, the lactose permease was found in the membrane fraction rather than as a soluble protein. Further analysis showed that the lactose permease assembled into the isolated membrane vesicles in a native and functional configuration. As shown for the potassium channel KcsA, the assembly of protein complexes in a cell-free system can be dependent on the lipid composition [9-11]. The higher protein yields of new cell-free protein expression systems [12] like the wheat germ system from CFS offer now promising options for large-scale production of membrane proteins [13].

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

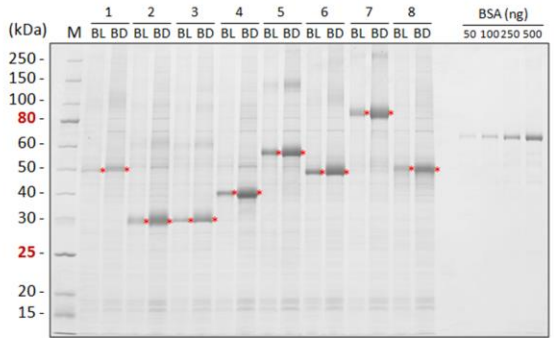
Application Note 2/2018: ProteoLiposomes BD Expression Kit

New BD reaction format

Among eukaryotic cell-free protein expression systems, wheat germ derived extracts [14] provide the highest protein yields as compared to extracts from rabbit reticulocytes or insect and human cell lines. The wheat germ system was used successfully for the preparation of many membrane proteins [15-19], including the preparation of a functional Shaker potassium channel [20], the preparation of isotope-labeled transmembrane proteins for targeted proteomics [21], and the preparation of monoclonal antibodies against different GPCRs [22, 23]. Many different lipids and detergents have been tested in combination with the wheat germ system [19, 24, 25]. However, asolectin, a mixture of natural, polyunsaturated phospholipids from soybeans, is used for decades for preparing proteoliposomes [26] and is presently probably the best option for working on a large variety of different membrane proteins.

To assist studies on membrane proteins, CFS offers the ProteoLiposome BD Expression Kit combining our High Performance WEPRO®7240 wheat germ system with lyophilized liposomes prepared from asolectin. After rehydration those liposomes can be directly used in the translation reaction, thus avoiding the use of any organic solvents involved in liposome preparations. After completion of the protein expression experiment, proteoliposomes can easily be isolated by a centrifugation step providing sufficient purity for many applications including immunization of animals for antibody production.

The ProteoLiposome BD Expression Kit uses the new BD ("bilayer and dialysis") reaction format, which was developed for high-yield preparation of proteoliposomes [23]. The BD reaction format combines our bilayer protein expression method with a dialysis reaction for a more efficient buffer exchange and feeding of the translation reaction with fresh amino acids and energy supply. At the same time, dialysis is used to remove inhibitors of the translation reaction thus allowing for high protein expression rates. Under these conditions, dialysis reactions can be maintained for up to three days without a need to exchange the feeding buffer.



Lane	Explanation
M	Molecular weight marker
BL	1 µl Bilayer reaction mixture
BD	1 µl BD reaction mixture
BSA	Reference Standard
*	Detected target protein

Figure 1: SDS-PAGE analysis of GPCRs expressed in a Bilayer and BD translation reaction. Equal amounts of the crude reaction mixtures were loaded along with a BSA standard for quantification.

During our tests on the expression of several membranes proteins, we found that the new BD reaction format yields up to four times the protein amounts of a standard bilayer reaction of the same size (Figure 1, and data shown in Table 1). The 2.5 ml standard BD expression reaction yields for example for the G Protein-Coupled Taste Receptor T1R1 over 500 µg of protein in the purified proteoliposome fraction. The protein yields for all proteins in the experiment were estimated from the SDS-PAGE shown in Figure 1 by reference to the BSA standard and are given in Table 1 below:

Lane #	Protein Name	Protein Yield (µg)	
		Bilayer Reaction (4 ml reaction)	Bilayer-Dialysis reaction (2.5 ml reaction)
1	CHRM2	78	163
2	GHSR	156	539
3	PTGER1	121	293
4	GHRHR	165	767
5	GPR56	236	669
6	FZD7	235	514
7	GABBR1	167	552
8	T1R1	161	520

Table 1: Comparison of protein yields from Bilayer and Bilayer-Dialysis translation reactions; data taken from SDS-PAGE shown in Figure 1.

As shown in Figure 2, BD reactions are easy to setup and require no further attention until the expression reaction is completed. The ProteoLiposome BD

Expression Kit provides all necessary reagents to perform six preparative 2.5 ml BD reactions. Additional reagents are provided with the kit to further allow for small-scale expression experiments for testing expression vectors prior to scaling up the reaction size; CFS can further provide a protocol to perform BD reactions on a 0.5 ml scale using the same kit format. The smaller 0.5 ml format can also be used for screening the expression of different membrane proteins into proteoliposomes.

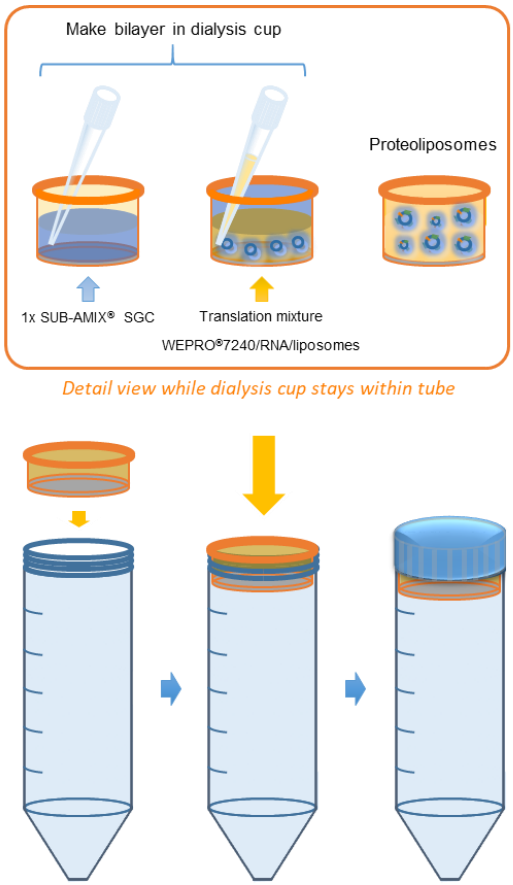


Figure 2: Setup of BD reaction. First place dialysis cup into 50 ml tube holding reaction buffer. Second, setup the bilayer translation reaction in the dialysis cup as shown in the upper panel. Then put lid onto tube with dialysis cup and conduct translation reaction for up to 3 days.

A membrane protein expression experiment on the BD format requires only few steps: The ORF ("open Reading Frame") for the protein of interest should be cloned into expression vector pEU-E01-MCS, which has a SP6 promoter to drive the RNA expression and a dedicated translation enhancer for the wheat germ system. After cloning, any new expression vector should first be tested for protein expression using the bilayer format without added liposomes described in the BD kit manual or using the 0.5 ml BD reaction format with added liposomes. Once protein expression from the vector has been confirmed, a 2.5 ml BD reaction can be set up for the preparation of proteoliposomes. The BD kit manual provides directions on how the proteoliposomes can be isolated from the crude reaction mixture after the completion of the translation reaction. Enriched proteoliposomes are collected in a PBS buffer for further analysis and storage. Protein expression should be confirmed by SDS-PAGE. Membrane proteins obtained from the proteoliposomes are commonly pure enough to conduct activity testing or to prepare antibodies.

Conclusion

With the ProteoLiposome BD Expression Kit, CFS wants to give scientists easy access to membrane proteins for further analysis and characterization. The lyophilized liposomes can be purchased separately, where we also offer biotinylated liposomes for use in screening assays. We hope these products will help studying membrane proteins for a better understanding of their role in life and possible use for the development of new therapies.

References

1. Hopkins, A.L. and C.R. Groom, *The druggable genome.* Nature reviews. Drug discovery, 2002. **1**(9): p. 727-30.
2. Overington, J.P., B. Al-Lazikani, and A.L. Hopkins, *How many drug targets are there?* Nature reviews. Drug discovery, 2006. **5**(12): p. 993-6.
3. Schlyer, S. and R. Horuk, *I want a new drug: G-protein-coupled receptors in drug development.* Drug discovery today, 2006. **11**(11-12): p. 481-93.
4. Zhang, D., Q. Zhao, and B. Wu, *Structural Studies of G Protein-Coupled Receptors.* Molecules and cells, 2015. **38**(10): p. 836-42.
5. Banerjee, R.K. and A.G. Datta, *Proteoliposome as the model for the study of membrane-bound enzymes and transport proteins.* Mol Cell Biochem, 1983. **50**(1): p. 3-15.
6. Chang, C.N., G. Blobel, and P. Model, *Detection of prokaryotic signal peptidase in an Escherichia coli membrane fraction: endoproteolytic cleavage of nascent f1 pre-coat protein.* Proceedings of the National Academy of

Sciences of the United States of America, 1978. **75**(1): p. 361-5.
7. Chang, C.N., P. Model, and G. Blobel, *Membrane biogenesis: cotranslational integration of the bacteriophage f1 coat protein into an Escherichia coli membrane fraction.* Proceedings of the National Academy of Sciences of the United States of America, 1979. **76**(3): p. 1251-5.
8. Ahrem, B., H.K. Hoffschulte, and M. Muller, *In vitro membrane assembly of a polytopic, transmembrane protein results in an enzymatically active conformation.* The Journal of cell biology, 1989. **108**(5): p. 1637-46.
9. van Dalen, A., et al., *Influence of lipids on membrane assembly and stability of the potassium channel KcsA.* FEBS letters, 2002. **525**(1-3): p. 33-8.
10. van Dalen, A., et al., *Efficient membrane assembly of the KcsA potassium channel in Escherichia coli requires the protonmotive force.* EMBO reports, 2000. **1**(4): p. 340-6.
11. van Dalen, A., et al., *Components required for membrane assembly of newly synthesized K+ channel KcsA.* FEBS letters, 2002. **511**(1-3): p. 51-8.
12. Carlson, E.D., et al., *Cell-free protein synthesis: applications come of age.* Biotechnology advances, 2012. **30**(5): p. 1185-94.
13. Klammt, C., et al., *Cell-free expression as an emerging technique for the large scale production of integral membrane protein.* The FEBS journal, 2006. **273**(18): p. 4141-53.
14. Harbers, M., *Wheat germ systems for cell-free protein expression.* FEBS letters, 2014. **588**(17): p. 2762-73.
15. Arimitsu, E., et al., *The ligand binding ability of dopamine D1 receptors synthesized using a wheat germ cell-free protein synthesis system with liposomes.* European journal of pharmacology, 2014. **745**: p. 117-22.
16. Fogeron, M.L., et al., *Wheat Germ Cell-Free Overexpression for the Production of Membrane Proteins.* Methods Mol Biol, 2017. **1635**: p. 91-108.
17. Nozawa, A., et al., *A cell-free translation and proteoliposome reconstitution system for functional analysis of plant solute transporters.* Plant & cell physiology, 2007. **48**(12): p. 1815-20.
18. Nozawa, A. and Y. Tozawa, *Incorporation of adenine nucleotide transporter, Ant1p, into proteoliposomes facilitates ATP translocation and activation of encapsulated luciferase.* Journal of bioscience and bioengineering, 2014. **118**(2): p. 130-3.
19. Periasamy, A., et al., *Cell-free protein synthesis of membrane (1,3)-beta-d-glucan (curdlan) synthase: co-translational insertion in liposomes and reconstitution in nanodiscs.* Biochimica et biophysica acta, 2013. **1828**(2): p. 743-57.
20. Jarecki, B.W., et al., *Function of Shaker potassium channels produced by cell-free translation upon injection into Xenopus oocytes.* Scientific reports, 2013. **3**: p. 1040.
21. Takemori, N., et al., *High-throughput synthesis of stable isotope-labeled transmembrane proteins for targeted transmembrane proteomics using a wheat germ cell-free protein synthesis system.* Molecular bioSystems, 2015. **11**(2): p. 361-5.
22. Hashimoto, Y., et al., *Engineered membrane protein antigens successfully induce antibodies against extracellular regions of claudin-5.* Sci Rep, 2018. **8**(1): p. 8383.
23. Takeda, H., et al., *Production of monoclonal antibodies against GPCR using cell-free synthesized GPCR antigen and biotinylated liposome-based interaction assay.* Scientific reports, 2015. **5**: p. 11333.
24. Fogeron, M.L., et al., *Wheat germ cell-free expression: Two detergents with a low critical micelle concentration allow for production of soluble HCV membrane proteins.* Protein Expr Purif, 2015. **105**: p. 39-46.
25. Rajesh, S., T. Knowles, and M. Overduin, *Production of membrane proteins without cells or detergents.* N Biotechnol, 2011. **28**(3): p. 250-4.
26. Kondrashin, A.A., et al., *Reconstitution of biological molecular generators of electric current. Inorganic pyrophosphatase.* European journal of biochemistry / FEBS, 1980. **113**(1): p. 219-22.

Follow us on Twitter (@CFSciences) for up-to-date information on new publications, products and the use of our expression system.