CellFree Sciences

The natural power of wheat driving science

High Performance Cell-Free Wheat Germ Protein Expression System

INSTRUCTION MANUAL

Asolectin Liposomes (lyophilized)

For use in combination with ProteoLiposome BD Expression Kit

Product Number(s): CFS-PLE-ASL

Version/date: Version 1.0_eng/March 2022

This product has a shelf life of 1 year being properly stored at -20°C.

CFS products are for research use only.

All our manuals are available for download in PDF versions on our homepage at: https://www.cfsciences.com/eg/. Work with electronic versions where possible to reduce paper waste.

ISO 9001 JQA-QMA15695 091

Our products are produced under a strict quality management system offering high-quality reagents including wheat germ extracts from wheat obtained by natural farming in Japan.

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Web: https://www.cfsciences.com/eg/ Follow us on Twitter: @CFSciences

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Important Information

Shipment and Storage

Our products are shipped on dry ice. While lyophilized liposomes are stable below room temperature, we recommend to kept them stored at -80°C right upon arrival. Rehydrate the liposome only when needed to perform an experiment. Avoid repeated freeze/thawing cycles of the rehydrated liposomes. Prepare aliquots of the rehydrated liposomes on first use if you want to keep on making more expression experiments later. Rehydrated liposomes may be stored at -80°C for up to 3 months.

Safety

Read the protocol carefully before starting the experiment. Do not drink or eat in the laboratory, and always wear gloves and a lab coat while working in the lab.

Wash hands before and after doing an experiment. If you get any reagent(s) in your eyes or on your skin, wash eyes or skin immediately with water. Although this kit does not contain any hazardous reagents, do not take any risk.

Note that cell-free protein expression experiments must be conducted under RNase-free conditions.

Safety Data Sheets (SDS) for our products can be downloaded from our homepage at:

https://www.cfsciences.com/eg/

Contact CellFree Sciences for further support and advice if you have any questions on the experiments described herein and materials provided with this product. Contact information is given at the end of this manual.

Other information

Refer to the manual of the ProteoLiposome BD Expression Kit (CFS-CPLE-BDL) for more information on how to use the liposomes in a cell-free protein expression experiment. CFS also offers biotinylated liposomes (CFS-ASL-BT) for use in screening experiments.

Introduction

Liposomes, artificially prepared spherical lipid vesicles, can be added to cell-free protein expression reaction for direct formation of proteoliposomes. Proteoliposomes are a convenient way to prepare and later study membrane proteins. Liposomes made from asolectin have been successfully used in the wheat germ cell-free protein expression system for studies on GPCRs, Transporters and other membrane proteins.

Asolectin is a mixture of phospholipids prepared from soybeans. It contains about equal amounts of lecithin, cephalin and phosphatidylinositol along with small amounts of other phospholipids and polar lipids.

Our liposomes are lyophilized for stable storage and easy reaction setup and are intended for use in combination with our wheat germ cell-free protein expression system. This product does not contain any reagents to conduct translation reactions nor are any buffers provided to rehydrate the liposomes.

Refer to our homepage or contact our support team for more information on how to use our cell-free protein expression system. The contact information is given at the end of the manual.

Kit Contents

The following tables summarize the reagents provided with this product:

Lyophilized liposomes (green box):

Item	Quantity	Amount	Vial
Asolectin Liposomes, lyophilized	6	10 mg	Brown glass vials

Refer to the table below for more information on how to handle and store the reagents:

Item	Description	Storage
Asolectin Liposomes, lyophilized	Lyophilized liposomes are provided in a specially sealed vial to avoid any air contact and are stable below room temperature. Rehydrated liposomes are for single use, and we recommend not to freeze/thaw the liposomes after rehydration. They may be stored at -80°C for 3 months.	-80°C

Required Reagents and Instruments

Consumable	Description
1x SUB-AMIX® SGC	Used for rehydration of liposomes. Sold separately.
Centrifuge	Suitable for 1.5 ml tube and 50 ml tube
Sonicator bath (optional)	Needed to prepare liposome solution

Protocol

Rehydration of lyophilized Liposomes

Perform the following steps to rehydrate the lyophilized liposomes. You require 1x SUB-AMIX® SGC translation buffer for rehydrating the lyophilized liposomes. We advise to sonicate the liposome solution when using the proteoliposomes for functional analysis. After sonification most liposomes will form a uniform single lamella. If you do not have a sonication apparatus, rehydrated liposomes can be used directly in the subsequent protein synthesis reaction. However, they are non-uniform in size and form multi-lamella. Sonication is also not needed if the proteoliposomes are later used for example as antigens in immunization experiments.

Preparation of liposomes without sonication step:

1. Open outer and inner cover of the vial containing the asolectin liposomes.

2. Slowly add 200 µl of 1x SUB-AMIX® SGC to the center of bottom of the vial.

3. Close the vial with inner cover and let it stay at room temperature for 10 min.

4. Mix the liposomes by vortexing (30 seconds to 1 minute). After vortexing, the solution will appear cloudy, and liposomes will be multi-dispersed having a non-uniform particle size.

5. Transfer the vial to a 50 ml tube and centrifuge them at 500x g for 1 min.

6. Take out the vial from 50 ml tube and transfer the rehydrated liposomes to a 1.5 ml tube for later use.

Preparation of liposomes with sonication step:

1. Open outer and inner cover of the vial containing the asolectin liposomes.

2. Slowly add 200 µl of 1x SUB-AMIX® SGC to the center of bottom of the vial.

3. Close the vial with inner cover and let it stay at room temperature for 10 min.

4. Mix the liposomes by vortexing (30 seconds to 1 minute). After vortexing, the solution will appear cloudy. Rehydrated liposomes are multi-dispersed and do not have a uniform particle size.

5. Transfer the vial to a 50 ml tube and centrifuge them at 500x g for 1 min.

6. Take out the vial from 50 ml tube and transfer the rehydrated liposomes to a 1.5 ml tube for later use.

7. Sonicate the liposome solution using a sonication apparatus until the solution becomes clear. The clear solution will have a yellowish color. For example, when using a Digital Sonifier equipped with a horn cup (BRANSON) the liposome solution becomes clear within 2 to 5 minutes at 20-30% output power. After sonification, the particle size of the liposomes in the solution commonly has a peak distribution at about

150 nm based on scattered light intensity measurement, and the liposomes should be close to a monodispersed distribution.

8. Centrifuge the clear liposome solution at 20,000g for 5 min to remove debris.

9. Use the supernatant for the subsequent protein synthesis.

If you do not use the rehydrated liposomes immediately, subdivide them into appropriate volumes in separate tubes and store them at -80°C after freezing with liquid nitrogen. Rehydrated liposomes are for single use (*1). You will require 100 µl of a 50 mg/ml liposome solution to setup a 2.5 ml BD protein expression reaction.

(Notes)

*1: Avoid multiple freeze-thawing cycles after rehydrated.

Troubleshooting

Contact the technical support of CellFree Sciences for more information and further help. The contact information is given on the last page of the manual.

Customer Information

Label License Policy

Our products are covered by the US patents Nos. 7838640, 7981617, 8734856, and equivalent pending or granted patents in other countries. Please contact us at tech-sales@cfsciences.com if you indent to make commercial use of our expression system.

Commercial use includes:

- Using the product as a raw material in manufacturing process.
- Using the product for offering paid services.
- Reselling the product or any of its components.

All our products are for RESEARCH USE ONLY. NOT FOR HUMAN OR ANIMAL THERAPEUTIC OR DIAGNOSTIC USE.

Trademarks

ENDEXT®, WEPRO® and SUB-AMIX® are registered trademarks of CellFree Sciences Co., Ltd.

Others

All product specifications and information in the manual may be changed without prior notice.

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

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