CellFree Sciences

The natural power of wheat driving science

High Performance Cell-Free Wheat Germ Protein Expression System

INSTRUCTION MANUAL

NanoDisc BD Kit

This kit provides reagents to prepare NanoDisc in cell-free protein expression experiments

Product Number(s): CFS-CNDE-BD

Version/date: Version 1.3_eng/July 2024

This Product has a shelf life of 1 year being properly stored at -80°C.

CFS products are for research use only.

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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Important Information

Shipment and Storage

Our products are shipped with dry ice. Wheat germ extracts are temperature sensitive and must always be kept frozen. Store kit at -80°C right upon arrival and only thaw reagents when needed. Avoid repeated freeze/thaw cycles. Prepare aliquots of the wheat germ extract on first use if you want to keep making more expression experiments later; refer to the protocol below on how much extract is needed per reaction. Do not freeze/thaw the wheat germ extract more than three times.

Safety

This kit can be used in a regular molecular biology laboratory. We strongly advise to work under RNase-free conditions. Refer to a laboratory handbook for more information on how to work under RNase-free conditions.

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.

Inform yourself about the necessary precautions for performing SDS-PAGE experiments using high voltage, and toxic chemicals in case you wish to prepare your own gels.

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.

For your convenience

CellFree Sciences provides short versions of our protocols (“Bench Notes”). Use these Bench Notes to setup your transcription and translation experiments at your workplace. They only contain the basic information needed for setting up the experiments. Use the checkmarks in the Bench Notes to assure that all pipetting steps have been completed correctly.

Introduction

Although nearly one-third of all eukaryotic genes encode membrane proteins, there are still many challenges in their functional and structural analysis. This is mainly due to the fact that membrane proteins can be toxic to cellular systems or form insoluble aggregates, and the complexity of expressing them in standard protein expression systems. CellFree Sciences Co., Ltd. has been providing BD (dialysis-bilayer) kits for synthesizing membrane proteins as proteoliposomes, which are used for antigen preparation and other purposes to prepare antibodies to membrane proteins.

Now, as additoinal research tool for membrane proteins, we offer the NanoDisc synthesis kit, a particle-like structure that can be manipulated in an aqueous environment by binding a phospholipid bilayer containing the target protein to the MSP (apolipoprotein A-1). Proteins in the NanoDisc are monodisperse in solution, which is expected to improve particle uniformity compared to proteoliposomes.

This kit is based on the BD reaction method developed for proteoliposome synthesis, and enables more efficient and higher quality NanoDisc. This method enables more efficient and higher quality synthesis of NanoDisc.

Kit Contents

The following tables summarize the reagents provided with this product: The reagents are shipped in following four boxes.

① NanoDisc BD Kit (white box):

Item	Quantity	Volume	Vial	Vial Color
● **Transcription Reaction**				
5xTranscription Buffer LM	1	240 µl	1.5 ml tube	Yellow
NTP Mix (25 mM)	1	120 µl	1.5 ml tube	Blue
RNase Inhibitor (80 U/µl)	1	15 µl	1.5 ml tube	Orange
SP6 RNA Polymerase (80 U/µl)	1	15 µl	1.5 ml tube	Green
● **Translation Reaction**				
WEPRO®7240ND	1	1000 µl	2.0 ml tube	Clear
Creatine Kinase (20 mg/ml)	1	20 µl	1.5 ml tube	Red
40xSUB-AMIX® SGC S-1	1	600 µl	1.5 ml tube	Purple
40xSUB-AMIX® SGC S-2	1	600 µl	1.5 ml tube	White
40xSUB-AMIX® SGC S-3	1	600 µl	1.5 ml tube	Brown
40xSUB-AMIX® SGC S-4	1	600 µl	1.5 ml tube	Gray

② ND Plasmid set (white box):

Item	Quantity	Conc. & Vol.	Vial	Vial Color
pEU-E01-MCS-TEV-SBP-C1 (SBP)	**1**	**1 mg/mL, 10 uL**	**1.5 ml tube**	**Green**
pEU-E01-MSP1E3D1 (MSP)	**1**	**1 mg/mL, 85 ul**	**1.5 ml tube**	**Purple**
pEU-E01-CLDN4-SBP (CLDN)	**1**	**1 mg/mL, 10 ul**	**1.5 ml tube**	**Pink**

③ SA Set (white box):

Item	Quantity	Volume	Vial	Vial Color
40xSUB-AMIX® SGC S-1	1	7 ml	15 ml tube	Purple
40xSUB-AMIX® SGC S-2	1	7 ml	15 ml tube	White
40xSUB-AMIX® SGC S-3	1	7 ml	15 ml tube	Brown
40xSUB-AMIX® SGC S-4	1	7 ml	15 ml tube	Gray

④ Asolectin liposome, lyophilized (green box):

Item	Quantity	Amount	Vial	Vial Color
Asolectin Liposome, lyophilized	6	10 mg	Amber glass vials	Amber

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

Item	Description	Storage
WEPRO®7240ND	WEPRO®7240ND wheat germ extract is temperature sensitive! Immediately after thawing the extracts place them on ice. Upon thawing for the first time, prepare aliquots for later use and store them at -80°C. Do not freeze/thaw the wheat germ extract more than three times. We recommend using liquid nitrogen to freeze the extracts.	-80°C
40xSUB-AMIX® SGC (S-1, S-2, S-3, S-4)	The 40xSUB-AMIX® SGC translation buffer is provided in four separate master mixes (S-1, S-2, S-3 & S-4) to avoid the precipitation of some amino acids. It is possible to prepare onetime a large volume of the 1xSUB-AMIX® SGC translation buffer, and to store aliquots at -80°C for later use. Do not mix the 40xSUB-AMIX® SGC translation buffers directly as some amino acids will precipitate. Avoid unnecessary freeze/thawing of the 1xSUB-AMIX® SGC translation buffer. You will need the 40xSUB-AMIX® SGC translation buffers provided in the Expression Reagent Kit and the separate buffers to perform 6 BD reactions on a 2.5 ml scale.	-80°C
5xTranscription Buffer LM	After thawing for the first time, divide 5xTranscription Buffer LM into appropriate aliquots convenient for later use. The buffer can be stored at -20°C.	-20°C
NTP Mix	The NTP Mix contains ATP, GTP, CTP, and UTP at a concentration of 25 mM. The NTP Mix can be stored at -20°C.	-20°C
SP6 RNA Polymerase	Provided in 50% glycerol, and can be stored at -20°C.	-20°C
RNase Inhibitor	Provided in 50% glycerol, and can be stored at -20°C.	-20°C
Creatine Kinase (*1)	Creatine Kinase is temperature sensitive, and we recommend to always use fresh enzyme preparations. The Creatine Kinase in the kit is provided at a concentration of 20 mg/ml. This concentration can be directly used to set up translation reactions on a BD reaction scale. The enzyme must be diluted with nuclease-free water to 1 mg/ml for setting up SMALL scale translation reactions for testing protein expression.	-80°C
Asolectin Liposome, lyophilized	Lyophilized asolectin liposomes are provided in a specially sealed vial to avoid any air contact and are stable at -80°C. Open outer and inner covers of the vial containing the asolectin liposomes. Slowly add 200 µl of 1x SUB-AMIX® SGC at the center of bottom of the vial. Close the vial with the inner cover and let it stay at room temperature for 10 min. Mix the liposomes by vortexing (30 seconds to 1 minute). Transfer the liposomes from the vials to a 50 ml tube and centrifuge them at 500x g for 1 min. Take out the vials from the 50 ml tube and transfer the rehydrated liposomes to a 1.5 ml tube. We advise to sonicate the liposome solution before use in a water bath until the solution becomes clear and the liposomes form uniform single lamella. The clear solution will have a yellowish color. Rehydrated liposomes are for single use, and we recommend not to freeze/thaw the liposomes after rehydration.	-80°C

*1: Creatine Kinase can be purchased from Sigma-Aldrich, Catalog No. 10127566001.

Required Reagents, Consumables, and Instruments

Consumable	Description
Nuclease-free water	DNase, RNase free. We DO NOT recommend DEPC treated water
Slide-A-Lyzer™ MINI Dialysis Devices, 10K MWCO	Thermo Scientific™ Slide-A-Lyzer™ MINI Dialysis Devices, 10K MWCO from Thermo SCIENTIFIC, Product No. 88404.
Incubator	Temperature range 15 to 37°C.
Centrifuge	Suitable for 1.5 ml tube and 50 ml tube
Sonicator bath (optional)	Needed to prepare liposome solution
Reagents & consumables for purification	
SDS-PAGE	Gel electrophoresis apparatus and power supply

Protocol

The NanoDisc BD Kit provides enough reagents to do all steps required to test the expression of your membrane protein of interest on a preparative scale (5 reactions). Contact CFS on available control vectors that can be used in a separate expression experiment. Preparative expression for the preparation of NanoDisc is done on a 2.0 ml BD reaction scale. And NanoDisc is synthesized by co-expression of mRNA for MSP synthesis and mRNA for target protein synthesis in the presence of liposomes, resulting in high quality crude NanoDisc with high yield and low protease content, one of the characteristics of wheat cell-free synthesis. After synthesizing crude NanoDisc, it will be purified by SBP-Tag, fused into target protein, based affinity chromatography.

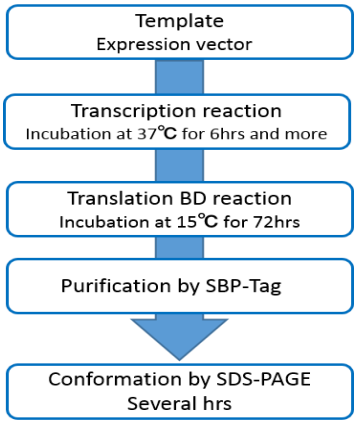
Successful protein expression should be confirmed before using any protein in your later experiments. Protein expression can be analyzed by SDS-PAGE to see whether a protein has been made. It can be helpful to compare your protein in a crude expression reaction mixture to a negative control reaction prepared without added expression vector. The negative control reaction will only show the background proteins in the wheat germ extract. We advise to use known amounts of a BSA standard in the SDS-PAGE experiment to estimate protein yields. As an alternative, protein expression can also be confirmed by Western blotting using an antibody against the target protein or an affinity tag. When working with small protein amounts, Western blotting and labeling methods offer more sensitive protein detection than protein staining in SDS-PAGE gels. Those methods further provide back-ground free-detection as commonly proteins in the wheat germ extract should not be recognized by a specific antibody, nor is there any background when labeling proteins during expression experiments.

Small -scales reaction

We recommend that before NanoDisc synthesis trials, you confirm your target protein can be synthesized by 227 µl small-scale reaction without liposomes.

Time Requirements

Refer to the flowchart below on the estimated time per reaction step.



Preparation of NanoDisc using BD Reaction Format

Transcription Reaction Using DNA Template

1. From each template, mRNA for MSP translation and mRNA for target protein translation are prepared separately
2. Thaw your template DNA before the experiment. You need 13 µg each of purified plasmid DNA for a 2.0 ml BD reaction scale (*1).
3. Take vials with 5xTranscription Buffer LM (yellow vial), NTP Mix (blue vial), RNase Inhibitor (orange vial) and SP6 RNA polymerase (green vial) out from storage at -80°C.
4. Thaw the reagents in a water bath at ~25°C. After thawing, spin-down the vials briefly to collect the entire volume at the bottom of the vial. Mix the reagents gently before use. Always keep reagents on ice.
5. Set up the transcription experiment as shown in the table. Then mix gently by pipetting up and down.

Reagents	Volume	Final Concentration
Nuclease free water*	74.8 µl	-
5xTranscription Buffer LM	26 µl	1x
NTP Mix (25 mM)	13 µl	2.5 mM
RNase Inhibitor (80 U/µl)	1.62 µl	1 U/µl
SP6 RNA Polymerase (80 U/µl)	1.62 µl	1 U/µl
Plasmid (circular DNA, 1.0 µg/µl) for MSP synthesis	13ul	100 ng/µl
Or		
Plasmid (circular DNA, 1.0 µg/µl) for Target protein synthesis	13 ul	100 ng/µl
Total	130 µl	

***Do not use DEPC treated water; we recommend using commercially available pure water.**

6. Incubate at 37°C for 6 hours and more in an incubator (*2).
7. After completion of the transcription reaction, leave the reaction mixture at room temperature until later use in the translation reaction. Do not cool the reaction mixture, nor store it on ice.
8. Optionally, you can confirm the mRNA quality after the transcription reaction by agarose gel electrophoresis taking out 0.5 µl from the reaction mixture for loading onto the gel. Refer to a cloning handbook for more information on how to perform RNA gel electrophoresis. Successful RNA expression reactions yield in multiple transcripts that can be seen on the gel. Do not use an RNA preparation if you see an RNA smear because it indicates that your RNA had been degraded.

(Notes)

*1: Plasmid DNA produced using a commercial DNA purification kit is commonly used in protein expression experiments. Do not use plasmid DNA from alkaline lysis without further purification.

*2: A white precipitate may occur during incubation. This is magnesium pyrophosphate, which will not interfere with the following translation experiment. Use the whole reaction mixture including the precipitate in the next step.

Preparation of Translation Buffer for BD Reaction

Prepare 48 ml of feeding buffer (1x SUB-AMIX® SGC) for the dialysis reaction and the translation reaction. This buffer is also needed to rehydrate the lyophilized liposomes (*1). Mix buffer components of SUB-AMIX® SGC according to the table below. Add the water first before adding the buffer components to avoid precipitations. Mix the final buffer after all reagents have been added.

Reagent	Volume	Final Concentration
Nuclease-free water	43.2 ml	
40x SUB-AMIX® SGC (S1)	1.2 ml	1x
40x SUB-AMIX® SGC (S2)	1.2 ml	1x
40x SUB-AMIX® SGC (S3)	1.2 ml	1x
40x SUB-AMIX® SGC (S4)	1.2 ml	1x
Total	48 ml	

Keep buffer on ice until use.

(Notes)

*1: Do not mix directly the 40xSUB-AMIX® SGC translation buffers; always add them nuclease-free water for dilution. Do not use the 1xSUB-AMIX® SGC translation buffer if you observe any precipitation.

Rehydration of lyophilized Liposomes

To setup a 2.0 ml BD reaction, one vial with lyophilized asolectin liposomes (*1) is needed.

Preparation of liposomes with sonication step:

1. Leave the vial in the room temperature for 10 minutes and open outer and inner covers of the vial containing the asolectin liposomes.
2. Slowly add 200 µl of 1x SUB-AMIX® SGC to the center of the bottom of the vial.
3. Close the vial with inner cover and let it stay at room temperature for 10 min.
4. Mix the liposome containing vial by vortexing for 30 seconds to 1 minute.
5. Place the vial in a 50 ml tube and centrifuge at 500 x g for 1 minute.
6. Take out the vial from the 50 ml tube and transfer liposome content in the vial to a 1.5 ml tube.
7. Sonicate the liposome content using a sonication apparatus until the solution becomes clear. The clear solution will have a yellowish color. **Since sonication conditions (power output, time, etc.) depend on the device, etc., an example is given below (it is advisable to review the conditions beforehand).**
   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% power output. 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. Further, centrifuge the clear liposome solution at 20,000g for 5 min to remove debris.
9. Use the supernatant for the subsequent protein synthesis.

Translation Reaction Using RNA Prepared from DNA Template

After completion of the transcription reaction, let the reaction mixture cool down to room temperature. Do not forcibly cool it down on ice or in a refrigerator. Resuspend the transcription mixture by pipetting gently up and down before use in translation reaction (*1).

Perform the following steps (per reaction) to set up the translation reaction:

1. Take the vial with WEPRO®7240 wheat germ extract (clear vial). Thaw the extract in a water bath at ~25°C. After thawing, briefly spin-down the vial with wheat germ extract to collect it at the bottom of the vial. Avoid excessive centrifugation of wheat germ extracts. Mix the extract gently before use. Keep wheat germ extract on ice at all time. After use, immediately freeze the wheat germ extract in liquid nitrogen and put it back into the freezer for storage at -80°C. The wheat germ extract losses its activity if not kept at -80°C!
2. Thaw Creatine Kinase (red vial) in a water bath at ~25°C. After thawing, briefly spin-down the vial with Creatine Kinase to collect it at the bottom of the vial. Mix the enzyme gently before use. We strongly recommend not to freeze/thaw Creatine Kinase as it will rapidly lose activity (*2).
3. Prepare "translation mixture" as indicated in the table below. Mix gently by pipetting up and down and avoid any bubbles.

Reagent	Volume	Final Concentration
mRNA for Target Protein	56 µl	1/4 vol.
mRNA for MSP Protein	111 µl	
1x SUB-AMIX® SGC	65 µl	
WEPRO®7240 (240 OD)	167 µl	60 OD
Creatine Kinase (20 mg/ml)	1 µl	40 µg/ml
Asolectin liposome (50 mg/ml)	100 µl	10 mg/ml
Total	500 µl	

Keep reaction mixture on ice until use.

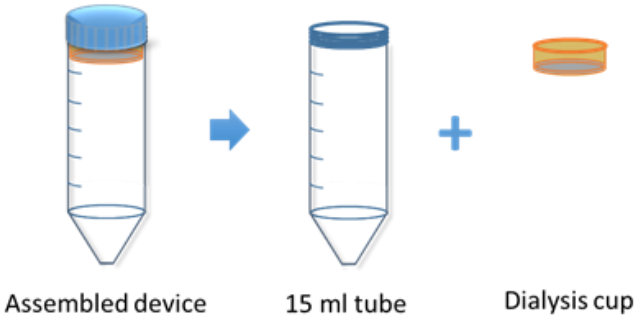
(Notes)

*1: If you notice a white precipitate after the transcription reaction, resuspend the precipitate by pipetting gently up and down before mixing with the wheat germ extract. There is no need to remove the precipitate.

*2: We recommend to always use fresh Creatine Kinase. Creatine Kinase is required for the energy supply of the translation reaction, and a loss of Creatine Kinase activity will reduce protein yields.

Preparation of BD Reaction

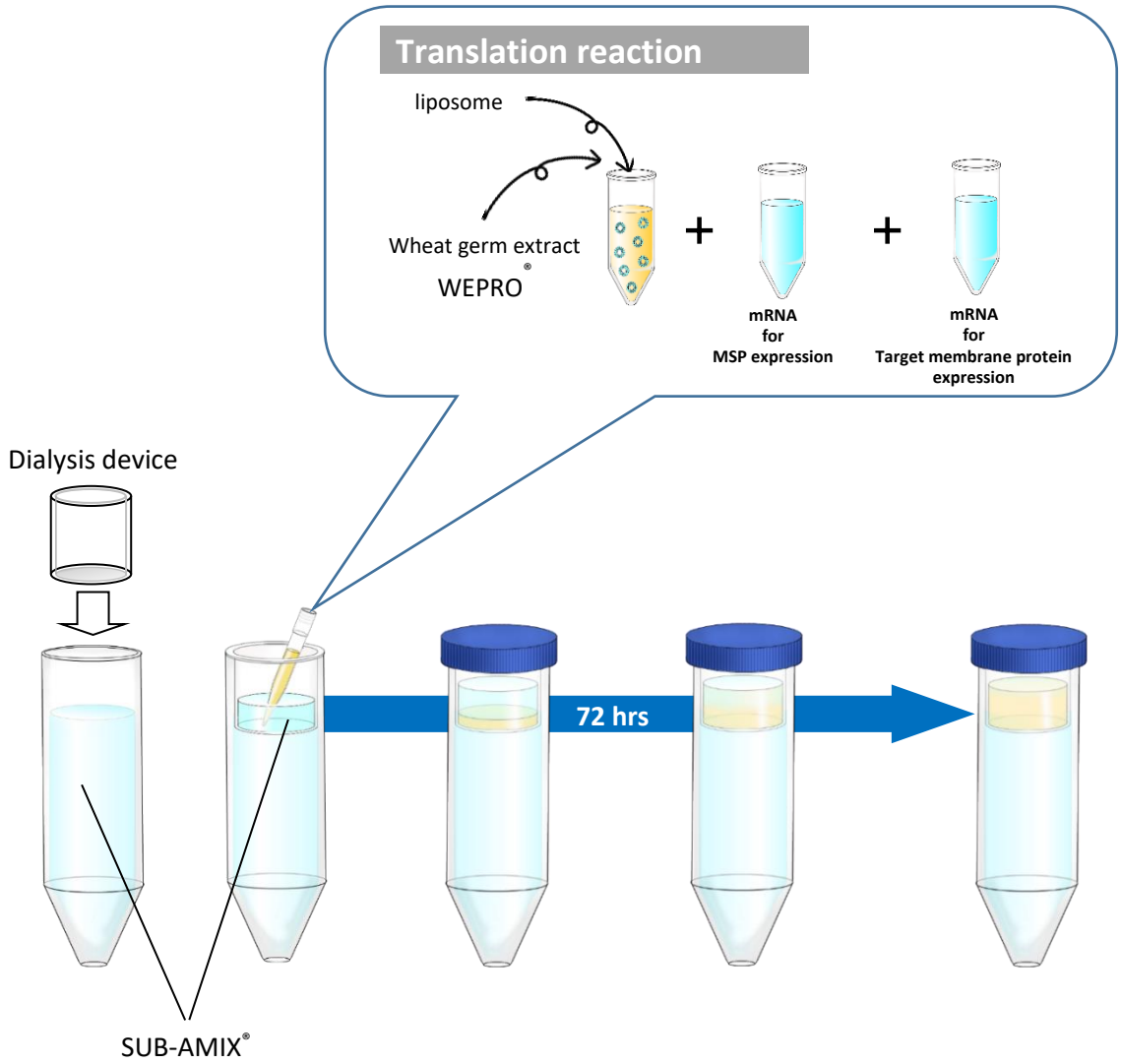
The BD reaction format combines the dialysis method with a bilayer reaction setup in the dialysis cup. For setting up a 2.0 ml BD reaction, we recommend using a Slide-A-Lyzer™ MINI Dialysis Devices, 10K MWCO from Thermo SCIENTIFIC, Product No. 88404. We have tested the reaction format using these dialysis cups, but similar products from other providers may work as well. Slide-A-Lyzer™ MINI Dialysis Devices, 10K MWCO comes with a 50 ml tube and a dialysis cup having a membrane at the bottom:



1. Remove the dialysis cup from Slide-A-Lyzer™ MINI Dialysis Devices, 10K MWCO and add 43 ml of 1x SUB-AMIX® SGC feeding buffer to the 50 ml tube.
2. Add 4 ml of nuclease-free water to the dialysis cup and rinse the dialysis membrane. Confirm there is no leakage from the membrane. Decant the water and shake the cup to remove remaining water. **Do not dry the membrane.**

3. Place the dialysis cup back to the 50 ml tube. Make sure the membrane in the dialysis cup is within the feeding buffer.
4. Add 2 ml of 1x SUB-AMIX® SGC to the dialysis cup. **Be careful not to damage the dialysis membrane.**

5. Set up a bilayer in the dialysis cup placed in the 50 ml tube with the feeding buffer.
6. Carefully transfer the translation mixture (500 µl) to the bottom of the dialysis cup containing 1x SUB-AMIX® SGC (2 ml) to form bilayer with the translation mixture in the lower layer and 1x SUB-AMIX® SGC in the upper layer. Bring the pipette tip close to the bottom of the dialysis cup, and slowly add the reaction mixture. Because of the higher density of the wheat germ extract, the reaction mixture will form a second layer below the reaction buffer. Do not disturb the layers when removing the pipette tip. **Be careful not to damage the dialysis membrane. Do not mix the reagents in the well by pipetting or any other means. Refer to the figure below for more information on how to set up the reaction.**
7. Close the 50 ml tube and tighten the cap on the tube. Be careful not to shake the tube with the bilayer reaction in the dialysis cup.
8. Place the tube in an incubator and incubate the translation reaction for up to 72 hours at 15°C. It is not necessary to exchange the feeding buffer during the incubation time.
9. We do not recommend changing the feeding buffer during the incubation to avoid disturbing the bilayer reaction. Note, the kit contains enough feeding buffer to do 6 times a 2.5 ml BD reaction, and no translation buffer is provided to change the buffer during the translation reaction. You can purchase additional SUB-AMIX® SGC (Product Number: CFS-SUB-AMX) from CellFree Sciences.



Flow of Bi-Layer & Dialysis (BD) reaction

Flow of Bi-Layer & Dialysis (BD) reaction

Illustration on how to setup of BD reaction: 1: Dialysis device comprising 50 ml tube, dialysis cup, and lid; 2: Prepare tube and add feeding buffer; 3: Insert dialysis cup into tube with feeding buffer; 4: Add feeding buffer into dialysis cup within the tube; 5: Setup bilayer reaction in the dialysis cup within the tube. Refer to drawings within the orange box for more details on how to place the translation mixture below the feeding buffer; 6: Put lid onto tube with dialysis cup and conduct translation reaction; 7: NanoDisc will be formed within the dialysis cup

Purification of NanoDisc

Crude NanoDisc can be purified by affinity chromatography with using SBP-Tag fused into target

Purification Buffer Preparation Notes:

Nanodisc is adversely affected by surfactants and glycerol, so these should not be added to the buffer.

- **Wash Buffer Preparation:**

Prepare buffers of pH 7-8 such as PBS, Tris, HEPES, etc., according to the intended use, with salt concentrations such as NaCl less than 500 mM; CFS uses 50 mM HEPES (pH 7.0) & 150 mM NaCl, or 50 mM Tris-HCl (pH 8.0) & 150 mM NaCl.

- **Elution buffer preparation:**

Add (well-suspended) stock solution of d-Desthiobiotin (e.g. d-Desthiobiotin, Cat. No. D1411, Merck (Sigma-Aldrich)) to the wash buffer to 5 mM, as Desthiobiotin has a low solubility, Because the solubility of desthiobiotin is low, the stock solution should be prepared by suspending it in MilliQ water or an appropriate buffer to a concentration of 200~500 mM, if all is dissolved, and then freezing it as a stock solution.

- **Streptavidin Sepharose HP (SA resin; Cytiva):**

Take the required amount, wash twice with an appropriate amount of MilliQ water, and then resuspend in an equal amount of wash buffer to make a 50% slurry.

1. Binding, washing and elution

Add an appropriate amount of SA resin (50% slurry) to the sample. Mix gently at 4°C for about 1 hour using a rotator. Mix gently at 4°C for about 1 hour using a rotator.

→Add an appropriate amount of SA resin (50% slurry) to the sample and mix gently at 4° C for about 1 hour using a rotator.

→For microdialysis multilayer synthesis, add 40ul (20ul resin) of 50% slurry, and for 500ul scale dialysis multilayer synthesis, add 400ul (200ul resin),

For 500ul scale dialysis multilayer synthesis, it is sufficient to add 400ul (200ul resin) each.

2. Collect the resin in an appropriate device (open column, spin column, etc.).

→Examples: Spin column; Ultrafree-MC-HV PVDF 0.45um (UFC30HV00; Millipore), Ultrafree-CL PVDF 0.45um (UFC40V25; Millipore), etc.

3. Wash the collected resin multiple times with an appropriate amount of washing buffer.

Add an appropriate amount of the wash buffer to your column of choice and spin down using 4000 g for 40 seconds. If there are remnants of the wash buffer that haven't been collected at the bottom of the column, repeat the spin down

→Since washing conditions depend on the purification scale, find appropriate conditions according to the scale.

CFS calculates the amount of washing buffer and the number of washing cycles according to the purification scale (amount of resin) using the following conditions.

$\left(\frac{Vwb}{Vresin}\right)^n > 30000$; V_{resin}: *amount of resin(ul)*、V_{wb}: washing buffer volume per washing *(ul)*、n: washing frequency

Calculation example: Resin volume 35ul (half of 50% slurry), buffer volume per wash 200ul, number of washes is 6

(200ul/35ul)^6 times = 34815 > 30000

4. Eluate your protein of interest.

Add an appropriate amount of the elution buffer, mix it with resin, let stand for 5 minutes and collect the eluate by spinning it down using 4000 g for 40 seconds. If there are remnants of the elution buffer that haven't been collected at the bottom of the column, repeat the spin down. Repeat 3~5 times.

Concentrate the eluate using a concentrator and exchange buffer until the Desthiobiotin concentration falls below 20uM.

→Repeat buffer exchange until Desthiobiotin concentration falls below 20uM, as Desthiobiotin interferes with UV quantification and SBP tag assay (reassociation to Streptavidin).

Store NanoDisc in PBS at -80 ˚C.

Confirmation of Protein Expression into NanoDisc

Proteins contained in the NanoDisc can be analysed by SDS-PAGE gel electrophoresis.
Load some 0.2 to 4 µl of the forgoing NanoDisc preparation per well for protein detection. **Take care not to boil the SDS–PAGE sample before loading.** We have observed that proteins may not enter the gel after they had been boiled in the SDS sample buffer (for example for a 2x SDS sample buffer containing 150 mM Tris-HCl (pH6.8), 1.2% SDS, 24% glycerol, 0.1% bromophenol blue, 4% 2-mercaptoethanol).

Additional Information

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.

Patent

CellFree Sciences' ENDEXT® technology and products are covered by US Patent Nos. 6869774, 7981617, 8734856 and other pending or equivalent patents

Customer Information

Product-related Information

All our products are for research purposes only; not for use in diagnostic testing and use in human.
Contact us for more information and help on the use of our products.

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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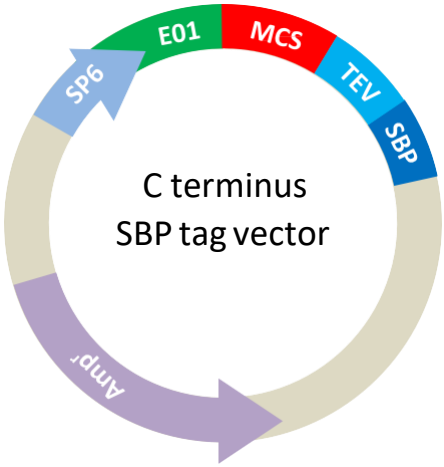
APPENDIX

Expression vector for NanoDisc

- pEU-E01-MCS-SBPtag-C1

1) MAP

Multiple cloning site information



pEU-E01-TEV-SBP-C1 (3884 bp)

↓*EcoRV* ↓*SpeI* ↓*XhoI* ↓*TEV cleavage site*

G ATA TCA CTA GTT CTC GAG GGC TCC GGC TCC GGC TCC GAG AAC CTC TAC TTC CAA GGC TCC GGC

Thr Ser Leu Val Leu Glu Gly Ser Gly Ser Gly Ser Glu Asn leu Tyr Phe Gln Gly Ser Gly

TCC GGC TCC GGC TCC GGC TCC GGC — SBP-tag — TGA **STOP**

Ser Gly Ser Gly Ser Gly Ser Gly

2) Sequence

SP6 Promotor
E01 Enhancer
MCS (EcoRV, Spe1, Xho1), adding ATG as start codon@N-site
TEV site
SBP-tag
Stop Codon

ATTTAGGTGACACTATAGAACTCACCTATCTCCCCAACACCTAATAACATTCAATCACTCTTTCCACTAACCACCTATCT
ACATCACCAAGATATCACTAGTTCTCGAGGGCTCCGGCTCCGGCTCCGAGAACCTCTACTTCCAAGGCTCCGGCTCCG
GCTCCGGCTCCGGCTCCGG ATGGACGAAAAGACCACCGGCTGGAGGGGCGGCCACGTGGTGGAGGGCCTCGCCG
GCGAGCTCGAACAACTCAGGGCCAGGTTAGAACACCACCCACAAGGCCAGAGGGAGCCATGAGGATCCATATATAG
GGCCCGGGTTATAATTACCTCAGGTCGACGTCCCATGGTTTTGTATAGAATTTACGGCTAGCGCCGGATGCGACGCCG
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