CellFree Sciences

The natural power of wheat driving science

High Performance Cell-Free Wheat Germ Protein Expression System

INSTRUCTION MANUAL

Protein Research Kits S16/G16/H16

These kits provide reagents to perform 16 cell-free protein expression experiments on a 226 µl bilayer format

Product Number(s): CFS-PRK-S16, CFS-PRK-G16, CFS-PRK-H16

Version/date: Version 1.0_eng/March 2019

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.

CellFree Sciences Co., Ltd., Yokohama Bio Industry Center, 1-6 Suehiro-cho, Tsurumi-ku, Yokohama, Kanagawa 230-0045, Japan
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. 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/thawing 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 is providing short versions of our protocols (“Bench Notes”). Use these Bench Notes to setup your transcription and translation experiments at your work place. 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

The WEPRO®9240 Protein Research Kits provide you with the latest version of our high-performance wheat germ cell-free protein expression system. Reagents in the Protein Research Kits are premixed and therefore allow to setup the transcription and translation reaction on a 226 µl scale by just a few pipetting steps. This is a very convenient format for performing multiple small-scale protein expression reactions. For further scaling up protein production, refer to our Core Kits or purchase our reagents individually to meet with your needs.

CellFree Sciences provides the premixed WEPRO®9240 wheat germ extracts in three different versions that can be used under identical reaction conditions. Besides the regular WEPRO®9240 extract for universal protein expression, we have a dedicated "WEPRO®9240G Version" for the preparation of GST-tagged proteins and a dedicated "WEPRO®9240H Version" for the preparation of His-tagged proteins. Wheat germ extracts of the WEPRO®9240G and WEPRO®9240H versions have been precleared to remove wheat proteins from the extracts that would otherwise bind to glutathione or Ni resins during the purification step.

CellFree Sciences provides dedicated expression vectors optimized for use with our wheat germ cell-free protein expression system. We recommend using our expression vectors for template preparation. It is also possible to prepare expression templates by PCR methods for rapid expression testing and high-throughput studies. Note that using linear DNA templates from PCR reactions will commonly lead to lower protein yields as linear DNA is less stable than circular plasmid DNA.

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 table summarizes the reagents provided with this product:

Item	Quantity	Volume	Vial	Vial Color
Transcription Premix LM*	16	18 µl	Strip of eight 0.2 ml PCR tubes	Blue
WEPRO®9240*	16	10 µl	Strip of eight 0.2 ml PCR tubes	Yellow
WEPRO®9240G*				Red
WEPRO®9240H*				Green
SUB-AMIX® SGC*	16	206 µl	Strip with eight wells	Clear
Aluminum seals	6	-	-	-

*Use entire volume within each vial per one reaction. The premixed reagents for the transcription and translation reactions are provided in strips holding 8 vials or wells. Cut off individual tubes or wells as needed before thawing the reagents. Never thaw reagents that are not needed for the experiment. Store all reagents at -80°C and avoid any unnecessary freeze/thawing. Wheat germ extracts will lose their activity when store above -80°C.

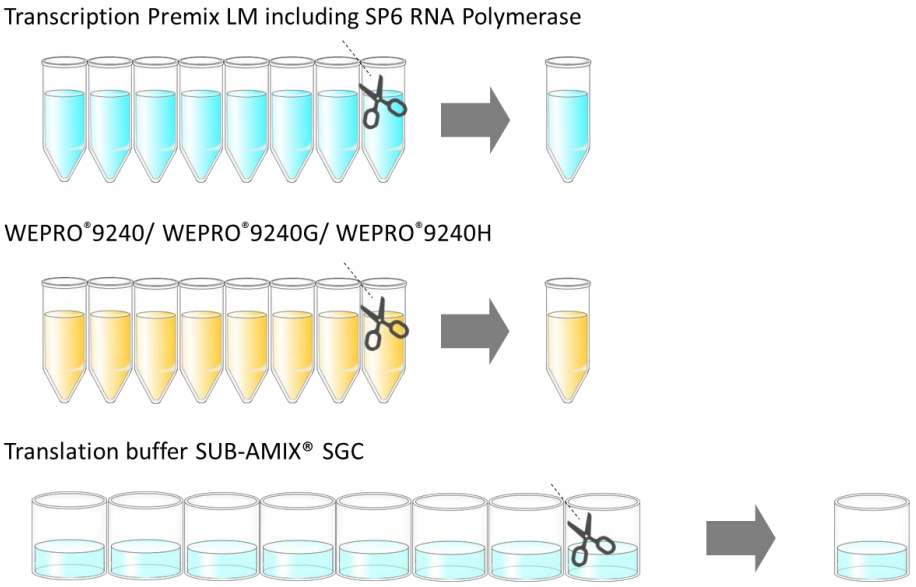


Illustration on handling of premixed expression reagents

Protocol

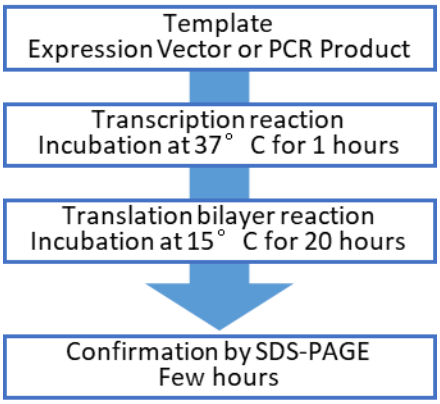
The WEPRO®9240 Protein Research Kits allow to perform protein expression reactions on a SMALL scale bilayer reaction scale of 226 µl. Bilayer translation reactions must be set up in flat-bottom vials. Therefore, the SUB-AMIX® SGC Translation Buffer included in this kit is already provided in flat-bottom vials that can be directly used to setup the protein expression reactions.

For first time use, we recommend including a positive control to make sure that the experiment has been set up correctly. Contact CFS on available control vectors that can be used in a separate expression experiment.

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 of the correct size 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 background 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.

Time Requirements

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



Transcription Reaction Using DNA Template

Per reaction perform the following steps to set up a transcription reaction:

1. Thaw your template DNA before the experiment. You need 2 µg of purified plasmid DNA at a concentration of 100 ng/µl (*1).
2. Take one vial with Transcription Premix LM (blue vial) per reaction from storage at -80°C (*2).

3. Thaw required number of Transcription Premix LM vials on ice. Keep the remaining vials at -80˚C. After thawing, spin the vials briefly to collect the entire volume at the bottom of the vial. Mix the reagent gently before use. Place reagents on ice and keep them cold at all times.
4. Add 2 µl of highly purified plasmid DNA (1.0 µg/µl) to each vial with the Transcription Premix LM as shown in the table below. Then mix gently by pipetting up and down.

Reagents	Working Volume	Final Concentration
Transcription Premix LM	18 µl	1 x
Plasmid (circular DNA, 1.0 µg/µl)	2 µl	100 ng/µl
Total	20 µl	

5. Incubate at 37°C for 1 hour in an incubator (*3).
6. Optionally, you can confirm the mRNA quality after the transcription reaction by agarose gel electrophoresis loading 0.5 µl of the reaction mixture. Refer to a cloning handbook for more information on how to perform RNA gel electrophoresis.

(Notes)

*1: Commonly plasmid DNA prepared by a commercial DNA purification kit is suitable for use in protein expression experiments. Do not use plasmid DNA from alkaline lysis without further purification.

*2: The strip holding the Transcription Premix LM vials can be cut into individual tubes by bending or cutting. Hold the vials firmly so that they do not pop open while separating them.

*3: 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.

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.

Per reaction perform the following steps to set up translation reaction:

1. Per reaction take from storage at -80°C storage a single vial with WEPRO®9240/ WEPRO®9240G/WEPRO®9240H (green vial) and a single well (clear well) containing SUB-AMIX® SGC (*1). Do not thaw unneeded vials and wells. Put the remaining vials and wells back into the freezer and store them at -80°C immediately. WEPRO®9240 loses its activity if not kept at -80°C!
2. Thaw reagents on ice. After thawing, briefly spin down each vial with WEPRO®9240 to collect the reagent at the bottom of the vial. Avoid excessive centrifugation of WEPRO®9240!
3. Resuspend SUB-AMIX® SGC by pipetting gently up and down in the well (*2).
4. Resuspend the transcription mixture by pipetting gently up and down (*3).
5. Prepare “translation mixture” by adding 10 µl of the “transcription mixture” containing the RNA template to the vial containing the WEPRO®9240 as indicated in the table below. Mix gently by pipetting up and down, avoid bubbles.

Reagents	Working Volume	Final Concentration
Transcription mixture (mRNA)	10 µl	0.5 vol.
WEPRO®9240/ WEPRO®9240G/WEPRO®9240H	10 µl	120 OD
Total	20 µl	-

6. Carefully transfer the translation mixture (20 µl) to the bottom of a single well containing SUB-AMIX® SGC (206 µl) to form bilayer with the translation mixture in the lower layer and SUB-AMIX® SGC in the upper layer. Refer to the figure at the end of this section on how to setup a bilayer reaction: Go with the pipette tip to the bottom of the well, and slowly release the translation mixture below the reaction buffer. Because of the yellowish color of the wheat germ extract, you can distinguish the translation mixture from the translation buffer in the well.

Reagent	Working Volume	Final Concentration
SUB-AMIX® SGC	206 µl	1 x
Translation mixture	20 µl	-
Total	226 µl	-

7. Seal the well with an aluminum seal included in the kit to avoid evaporation (*4).
8. Incubate at 15˚C for 20 hours in an incubator. Be careful that the well stably stands on a flat surface.
9. After completion of the translation reaction, mix the bilayer reaction gently by pipetting up and down.

(Notes)

*1: The strips with the WEPRO®9240 vials and SUB-AMIX® SGC wells can be split into individual vials or wells by bending or cutting. Hold the vials with the WEPRO®9240 firmly so that they do not pop open while separating them.
*2: Take particular care to keep the wells with SUB-AMIX® SGC upright at all times. They easily flip over, which would disturb the bilayer.
*3: If you notice a white precipitate after the transcription reaction, resuspend the precipitate by pipetting gently up and down before mixing with WEPRO®9240. There is no need to remove the precipitate.
*4: Cut off aluminum seals of an appropriate size to cover the wells. Peel off the brown cover on the back of the seals and glue the seal onto the well. Press down the seal onto the well to make sure it covers the entire surface of the well. The seal can easily be removed after the completion of the reaction by simply pulling it up. Save the remaining seals for later use. Be careful not to disturb the bilayer while placing the seal onto the well.

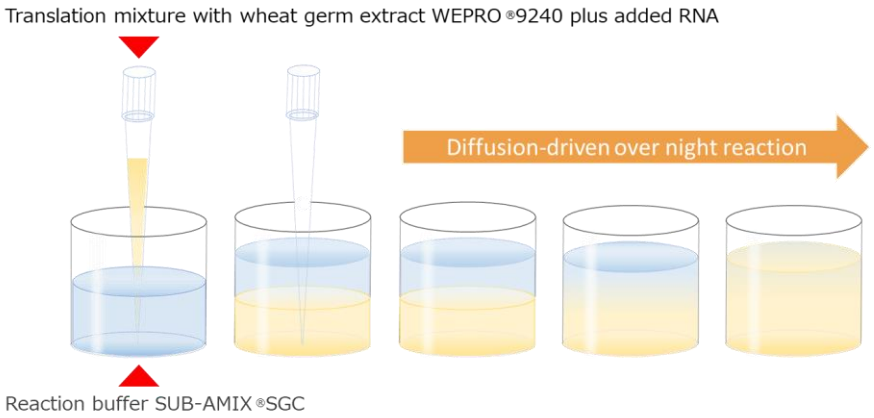


Illustration on how to setup bilayer reaction

Troubleshooting

The protein expression experiments require correct and accurate pipetting during reaction setup. Any mistake in the volumes added to the reactions, mixing the reagents, or forgetting any of the reagents will lead to wrong results. Therefore, carefully check the label for each reagent prior to starting the pipetting step.

- The experiment must be done under RNase-free conditions as any loss of the RNA template will prevent protein expression.
- Mark in your protocol each pipetting step you have completed.
- Change the pipetting tip after each pipetting step. Do not use the same pipetting tip to pipette different reagents or reaction mixtures. Always change the pipetting tip after use.
- Leaving out the plasmid template will always yield negative results. The same applies if there is a mistake in the expression vector, e.g. leaving out the starting ATG, forgetting a stop codon, or having a frame shift error.
- Confirm that your expression vector is correct and has a start and stop codon in line with the reading frame for the protein. Refer to our vector maps on more information on suitable sequencing primers to confirm the sequence of your expression vector.
- Confirm the DNA and RNA quality if the protein yields are low. Low RNA yields during the transcription reactions will also reduce the protein yields. Perform a phenol/chloroform extraction on the template DNA if RNA yields are low and ensure working under RNase-free conditions. An OD260/280 ratio of ~1.8 for your plasmid DNA preparations is commonly considered as pure enough for use in protein expression experiments. Lower ratios may indicate remaining proteins and/or other contaminations absorbing near 280 nm. Note that the actual values for the OD260/280 ratio can vary from vector to vector as the actual OD values depend also on the nucleotide composition of your DNA vector. It is important to confirm the OD260/280 ratio for your vector DNA before use in protein expression experiments because low DNA purity prevents RNA and protein expression.
- For the translation reaction, do not mix the two layers during setup of the bilayer translation reaction. Mixing both layers will sharply reduce the protein yields of a 20-hour translation reaction as the reaction will run dead within few hours. A slow mixing of both layers is required to maintain the translation reaction for up to 20 hours.
- It is possible to work with linear templates in cell-free protein expression experiments, which can be easily prepared by PCR methods. Working with PCR products, however, can reduce protein yields. PCR products are used to quickly find the best expression construct or to test expressing different protein fragments. If the yields obtained with PCR products are too low, it may be worthwhile to prepare an expression vector already for doing the test expression experiments. Always use expression vectors for large-scale protein production to have stable and reproducible conditions.
- If the protein is not expressed, check reaction conditions, reagents and DNA template in a small-scale expression reaction to confirm their integrity. If the results are unclear, you can check the performance of the transcription and translation reactions separately to narrow down the problem. Use a positive control vector to make sure that all reagents work, and the experiment is done correctly. Reconsider the design of your expression template to improve protein yields if all the forgoing steps do not explain low protein yields.

- Make sure that the wheat germ extract was always keep frozen before use. Avoid repeated freeze/thawing; it will inactivate the extract.
- Store wheat germ extract at -80°C; storage at higher temperature will lead to low activity or even total loss of activity. Follow clearly the recommendations on reagent storage and handling.
- Keep all fractions during protein purification until you have confirmed the recovery of the purified proteins. If you are not able to recover the protein from the resin during the purification experiment, check whether the protein can be found in the flow through or the washing fractions.
- Some proteins may have special requirements and do not express well under standard conditions. Gather information on your target protein before the expression experiments to see whether additional considerations are needed. Contact us for more information on how to modify cell-free protein expression experiments.

Additional Information

Certain proteins may require changes to the expression reactions, where we have dedicated expression kits to prepare membrane proteins in the presence of added lipids or to prepare isotope-labeled proteins for use in MS or NMR studies. We can provide more information on the use of other additives such as detergents or ions in our cell-free protein expression system. Visit the homepage of CellFree Sciences for more information on other products and how to use our protein expression system.

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

By opening the cap of any of the reagents provided with this product, the buyer of the product is agreeing to be bound by the terms of the following Label License Policy. CellFree Sciences' ENDEXT® technology and products are covered by US Patent Nos. 6905843, 6869774, 7838640 and 7981617, and other pending or equivalent patents. The purchase of the products conveys to the buyer the non-transferable right to use the purchased products and components of the products in research conducted by the buyer. The buyer cannot sell or otherwise transfer (a) the products (b) their components (c) materials made using the products or their components to a third party or otherwise use the products or their components or materials made using the products or their components for commercial purposes. The buyer may transfer information or materials made through the use of the products to a scientific collaborator, provided that such transfer is not for any commercial purposes, and that such collaborator agrees (a) not to transfer such materials to any third party, and (b) to use such transferred materials and/or information solely for research and not for commercial purposes. For information on purchasing a license to products for purposes other than research, contact the Intellectual Property Department of CellFree Sciences at the address shown at the end of this manual.

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Others

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

Contact

CellFree Sciences Co., Ltd.
Yokohama Head Office
Yokohama Bio Industry Center, 1-6 Suehiro-cho, Tsurumi-ku, Yokohama, 230-0045 JAPAN
TEL：+81-45-345-2625 FAX：+81-45-345-2626
E-mail: tech-sales@cfsciences.com

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