CellFree Sciences

The natural power of wheat driving science

High Performance Cell-Free Wheat Germ Protein Expression System

INSTRUCTION MANUAL

WEPRO7240/WEPRO7240G/WEPRO7240H Core Kit

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

Product Number(s): CFS-C7, CFS-C7G, CFS-C7H

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This Product is for research use only.

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

Our products are produced under a strike 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 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®7240 Series Core Kits provide you with the latest version of our high-performance wheat germ cell-free protein expression system. This manual describes protocols to perform *in vitro* transcription and translation experiments at three different reaction scales using a bilayer reaction format for protein expression. CellFree Sciences provides WEPRO®7240 wheat germ extracts in three different versions that can be used under identical reaction conditions. Besides the regular WEPRO®7240 extract for universal protein expression, we have a dedicated "WEPRO®7240G Version" for the preparation of GST-tagged proteins and a dedicated "WEPRO®7240H Version" for the preparation of His-tagged proteins. Wheat germ extracts of the WEPRO®7240G and WEPRO®7240H 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
WEPRO®7240/WEPRO®7240G/ WEPRO®7240H Extract	1	1000 µl	2.0 ml tube	Clear
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	Green
SP6 RNA Polymerase (80 U/µl)	1	15 µl	1.5 ml tube	Orange
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

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

Item	Description	Storage
WEPRO®7240/ WEPRO®7240G/ WEPRO®7240H	WEPRO®7240/WEPRO®7240G/WEPRO®7240H wheat germ extracts are 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 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. Prepare 19.8 ml of nuclease-free water and add 550 µl of each master mix to the water. 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.	-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 MEDIUM and LARGE scale. The enzyme must be diluted with nuclease-free water to 1 mg/ml for setting up SMALL scale translation reactions.	-80°C

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

Protocol

The WEPRO®7240 Series Core Kits allow to perform protein expression reactions on different reaction scales. In this manual, we refer to a SMALL reaction scale of 227 µl (up to 100 reactions), a MEDIUM reaction scale of 1.2 ml (up to 20 reactions), and a LARGE reaction scale of 6 ml (up to 4 reactions). The reaction size is scalable and therefore other reaction sizes may also be used.

Note, the reagents provided with this kit can also be used to setup MEDIUM size reactions on a 1.2 ml scale (up to 16 reactions) and LARGE size reactions on a 6 ml scale (up to 3 reactions) on CellFree Sciences' fully automated Protemist DTII protein synthesizer. Follow the manuals provided with the instrument for more details on how to use the reagents on the Protemist DTII.

Bilayer translation reactions must be set up in flat-bottom vials! While individual flat-bottom vials are available, multiple reactions can be set up in multi-well plates. Multi-well plates are available with

different dimensions to match the translation reaction size. It is preferable to have a larger surface between both layers to achieve higher protein yields.

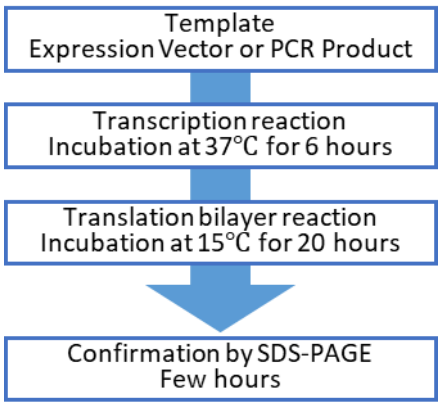
Plate Size	Well Volume	Reaction Size	Name in Manual
96 wells	~ 360 µl	227 µl	SMALL
24 wells	~ 3.4 ml	1.2 ml	MEDIUM
6 wells	~ 16.8 ml	6 ml	LARGE

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 on one of the reaction sizes:

1. Thaw your template DNA before the experiment. You need 1 µg (SMALL), 5 µg (MEDIUM) or 25 µg (LARGE) of purified plasmid DNA depending on the reaction scale (*1).
2. Take vials with 5xTranscription Buffer LM (yellow vial), NTP Mix (blue vial), RNase Inhibitor (green vial) and SP6 RNA polymerase (orange vial) out from storage at -80°C.

3. Thaw the reagents in a water bath at ~25°C. After thawing, spin 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.
4. Set up transcription experiment as shown in the table below for three different reaction sizes. Then mix gently by pipetting up and down.

Reagents	Volume SMALL	Volume MEDIUM	Volume LARGE	Final Concentration
Nuclease free water*	5.75 µl	28.8 µl	143.8 µl	-
5xTranscription Buffer LM	2 µl	10 µl	50 µl	1x
NTP Mix (25 mM)	1 µl	5 µl	25 µl	2.5 mM
RNase Inhibitor (80 U/µl)	0.125 µl	0.63 µl	3.13 µl	1 U/µl
SP6 RNA Polymerase (80 U/µl)	0.125 µl	0.63 µl	3.13 µl	1 U/µl
Plasmid (circular DNA, 1.0 µg/µl)	1 µl	5 µl	25 µl	100 ng/µl
Total	10 µl	50 µl	250 µl	

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

5. Incubate at 37°C for 6 hours in an incubator (*2).
6. After completion of the transcription reaction, leave reaction mixture at room temperature until later use in the translation reaction. Do not cool the reaction mixture, nor store it on ice.
7. 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: 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: 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. Resuspend the transcription mixture by pipetting gently up and down before use in translation reaction (*1).

Per reaction perform the following steps to set up translation reaction. Make sure to use information matching the transcription reaction size used in the previous step:

1. Take the vial with WEPRO®7240/WEPRO®7240G/WEPRO®7240H wheat germ extract (clear vial). Thaw the extract in a water bath at ~25°C. After thawing, briefly spin 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 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.

Reagents	Volume SMALL	Volume MEDIUM	Volume LARGE	Final Concentration
Transcription Reaction	10 µl	50 µl	250 µl	1/2 volume
Creatine Kinase (20mg/ml)	**0.8 µl***	0.2 µl	1 µl	40 ng/µl
WEPRO®7240/WEPRO®7240G/WEPRO®7240H Extract	10 µl	50 µl	250 µl	120 OD/ml
Total	20.8 µl	100.2 µl	501 µl	

***Dilute Creatine Kinase stock solution to 1 mg/ml with RNase free water when used for SMALL scale reaction setup!**

4. Prepare 1xSUB-AMIX® SGC translation buffer and add the required amount of buffer to a flat-bottom vial or well. Refer to the table below on the required volumes for the different reaction sizes (*3).

Reagents	Volume SMALL	Volume MEDIUM	Volume LARGE	Final Concentration
Translation Mixture	20.8 µl	100.2 µl	501 µl	-
1xSUB-AMIX® SGC	206 µl	1.1 ml	5.5 ml	1x
Total	227 µl	1.2 ml	6 ml	

5. Carefully transfer the translation mixture from step 3 to the bottom of a single vial or well containing 1xSUB-AMIX® SGC translation buffer to form the bilayer reaction with the translation mixture in the lower layer and the 1xSUB-AMIX® SGC translation buffer in the upper layer. Refer to figure at the end of this section on how to setup a bilayer reaction: Go with the pipette tip to the bottom of the vial or well, and slowly release the translation mixture below the 1xSUB-AMIX® SGC translation buffer. Because of the yellowish color of the wheat germ extract, you can distinguish the translation mixture from the translation buffer. The wheat germ extract has a higher density than the buffer and therefore will form easily the lower layer.
   Do not mix the reagents in the vial or well by pipetting or any other means! It will reduce the yield of the reaction.
6. Close the vial or seal the well to avoid evaporation.
7. Incubate at 15˚C for 20 hours in an incubator. Be careful that the vial stably stands on a flat surface.
8. After completion of the translation reaction, mix the bilayer reaction gently by pipetting up and down.
9. Place reaction mixture on ice to protect your protein before later use.

10. Commonly some 2 to 3 µl of the crude translation reaction mixture are enough to detect the expressed protein on SDS-PAGE. It can be helpful to load different amounts of the crude reaction mixture to get a better estimation of the protein yields.

(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.

*3: 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.

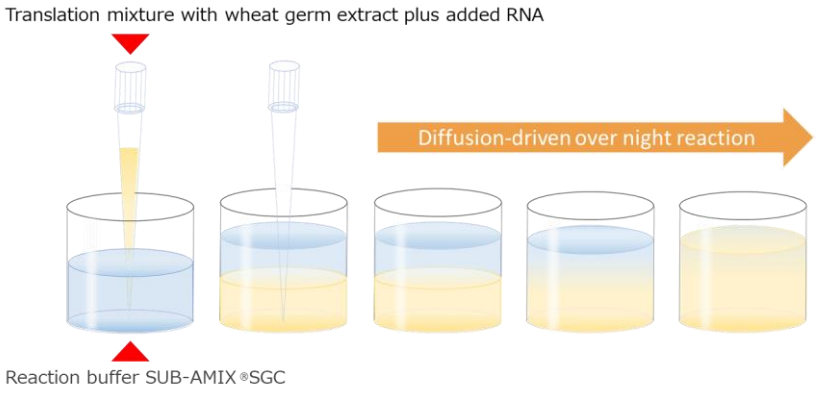


Illustration on how to setup bilayer reaction

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.
- We recommend to always using fresh Creatine Kinase. Do not freeze/thaw Creatine Kinase as it will rapidly lose activity. Creatine Kinase is required for the energy supply of the translation reactions, and a loss of Creatine Kinase activity will reduce protein yields.
- 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.

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