Continuous Protein Production

Protocol for Wheat Germ Cell-free Protein Expression

by

Dialysis Method

Manual version 1.1

Table of Content

1. INTRODUCTION

The wheat germ cell-free protein expression system has a very high protein translation activity that can be maintained over several days. While we recommend the bilayer reaction format for fast and convenient protein expression on a small scale (refer to our Protein Research Kits), a continuous supply of reagents by a feeding buffer in combination with removal of inhibitors is preferable for scaling up protein expression reactions to a preparative scale. To conduct such experiments manually, a dialysis-based approach can be easily setup without requirements for any special lab equipment as for example the Protemist XE Protein Synthesizer from CellFree Sciences for automated large-scale protein production.

This protocol describes protein synthesis using high activity wheat germ extracts from CellFree Sciences of the WEPRO7240 families and a dialysis method for the translation reaction. Using 0.5 ml of wheat germ extract in a 3 ml reaction, about 9 mg of crude protein (determined by the expression of GFP) can be prepared although the actual protein yield for your protein of interest may vary.

2. EXPERIMENTAL OUTLINE

This protocol follows our standard procedure, where in a first reaction step RNA is prepared from a DNA template by the means of the SP6 RNA polymerase. You can keep on using the same expression constructs in any pEU vector that had been used in other reactions with our protein expression system. In the second reaction step, the RNA is used in protein expression. The protein expression reaction is performed in a dedicated dialysis bag, which is place in a container filled with the feeding buffer. Proteins prepared by the dialysis methods can be further analyzed and purified by standard methods.

3. REAGENTS AND MATERIALS

3.1 Reagents available from CellFree Sciences

Product Name	Catalog #	Description	Storage
WEPRO®7240/ WEPRO®7240H/ WEPRO®7240G	CFS-WGE-7240/ CFS-WGE-7240H/ CFS-WGE-7240G	WEPRO®7240/7240H/7240G (wheat germ extract) are sensitive to temperature and vibration. Immediately after thawing under running water, place the reagent on ice. Upon thawing for the first time, separate the portion that is not used immediately, and to avoid multiple freeze-thawing exposures, subdivide it into appropriate aliquots in separate containers. Store them at -80°C for later use. Do not subject it to 3 or more freeze-thawing cycles. After the third freeze-thawing cycle, it is possible that protein synthesis activity decreases, the degree of which depends on the way of handling. Use of liquid nitrogen is recommended for re-freezing. When using the reagent, mix it gently by pipetting several times.	-80°C

		Avoid bubbling.	
SUB-AMIX® SGC (S1, S2, S3, S4)	CFS-SUB-SGC	This product consists of a set of 4 reagents (S1, S2, S3, S4) provided at 40x concentration. Store all 4 reagents at -20°C or below. No change in their reaction efficiency has been observed after 10 freeze-thawing cycles. To prepare 124 ml of 1x SUB-AMIX® SGC mixture, add 3.1 ml each of S1 through S4 to 112 ml of nuclease-free water while agitating the latter. If 4 reagents are mixed first, precipitation may occur. Once it happens, it takes time to dissolve the precipitates. To avoid multiple freeze-thawing cycles, subdivide 1x SUB-AMIX® SGC buffer into appropriate aliquots in separate containers and store them at -80°C. Do not subject 1x SUB-AMIX® SGC mixture to multiple freeze-thawing cycles. Decrease in the reaction efficiency may occur, the degree of which depends on the way of handling.	-20°C or -80°C
5x Transcription Buffer LM	CFS-TSC-5TB-LM	After thawing, subdivide 5x Transcription Buffer LM into appropriate aliquots convenient for your use. It has been confirmed that the freeze-thawing cycle for this product can be repeated up to 10 times.	-20°C
NTP Mix	CFS-TSC-NTP	ATP, GTP, CTP, and UTP in this NTP Mix have all been prepared at a concentration of 25 mM. After thawing, subdivide the NTP Mix into appropriate aliquots convenient for your use. It has been confirmed that the freeze-thawing cycle for this product can be repeated up to 10 times.	-20°C
SP6 RNA Polymerase	CFS-TSC-ENZ-S	Ready to use, buffer contains 50% glycerol.	-20°C
RNase Inhibitor		Ready to use, buffer contains 50% glycerol.	-20°C

Reagent use:

Product Name	Product Content	Volume per reaction	Number of reactions
SP6 RNA Polymerase	30 µl	13.2 µl	2
RNase Inhibitor	30 µl	13.2 µl	2
5xTranslation Buffer LM	210 µl	1000 µl	4
NTP Mix	105 µl	1000 µl	9
SUB-AMIX® SGC	12.5 ml	3.1ml	4
WEPRO7240	1 ml	0.5 ml	2

3.2 Reagents to be prepared by user

Reagent	Description
Nuclease free water	Remove DNases and RNases. We DO NOT recommend homemade DEPC treated water.
Creatine Kinase	Creatine Kinase can be purchased from Roche Applied Science, Catalog No. 10127566001. Dissolve it with nuclease-free water to make a 20 mg/ml stock solution. For convenience, subdivide the solution into smaller aliquots and store them at -80°C. Avoid multiple

	freeze-thawing cycles. Otherwise, the activity of Creatine Kinase may decrease.

3.3 Materials required

Consumable	Description
Dialysis cassette	Slide-A-Lyzer Dialysis Cassettes, gamma-irradiated, 10K MWCO, 3mL, from Thermo SCIENTIFIC, Product No. 66455.
Syringe	5 ml, sterilized.
Needle	18 gauge, sterilized.
Container	Container for dialysis reaction. Size; approx.7 cm x 11 cm x 4 cm. See section 4.2.
Incubator	Temperature range 15 to 37°C.
Shaker	Should fit into incubator.

4. PROTOCOL

For your safety:

Do not drink or eat in the laboratory. Do take precautions to work under RNase free conditions following standard lab procedures. Ware gloves and a lab coat at all times, and keep reagents on ice will setting up the reactions.

Wash hands before and after doing an experiment. If you have any reagent 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.

Read this protocol carefully before starting the experiment. Contact CellFree Sciences for further support and advice if you have any questions on the experiments described here and materials available from CellFree Sciences.

4.1 Transcription reaction

Prepare a 1,050 µl transcription mixture on ice according to the table below. Mix the reaction mixture gently by pipetting up and down before starting the incubation. Incubate reaction at 37°C for 6 hours. Shorter reaction times can reduce the protein yields.

Reagent	Volume	Final Concentration
Nuclease-free water	604 µl	-
5x Transcription Buffer LM	210 µl	1x
NTP Mix (25 mM)	105 µl	2.5 mM
RNase Inhibitor (80 U/µl)	13.2 µl	1 U/µl
SP6 RNA Polymerase (80 U/µl)	13.2 µl	1 U/µl
Plasmid (circular DNA, 1 µg/µl)	105 µl	100 ng/µl
Total	1,050 µl	

4.2 Preparation of feeding buffer SUB-AMIX® SGC

Prepare 124 ml of 1x SUB-AMIX® SGC according to the table below and mix gently. Transfer 120 ml of the 1x SUB-AMIX® SGC to a container. It will be used as the feeding buffer for the dialysis reaction. Prepare fresh 1x SUB-AMIX® SGC each time you want to replace the feeding buffer. We do not recommend to store 1x SUB-AMIX® SGC over several days while do the experiment.

Reagent	Volume	Final Concentration
Nuclease-free water	112 ml	
40x SUB-AMIX® SGC (S1)	3.1 ml	1x
40x SUB-AMIX® SGC (S2)	3.1 ml	1x
40x SUB-AMIX® SGC (S3)	3.1 ml	1x
40x SUB-AMIX® SGC (S4)	3.1 ml	1x
Total	124 ml	

Transfer 120 ml of 1x SUB-AMIX® SGC to a container as shown below.

4.3 Translation reaction mixture

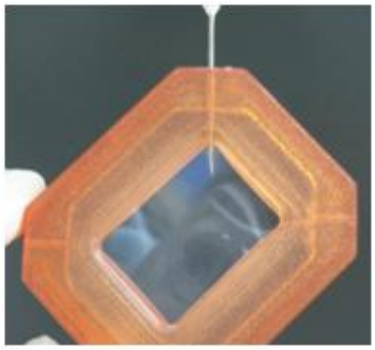
Set up a 3 ml translation reaction following the directions of the table below. Keep regents on ice at all times. Mix reaction mixture gently by pipetting by pipetting up and down. Avoid air bubbles.

Reagent	Volume	Final Concentration
mRNA	1 ml	1/3 vol.
1x SUB-AMIX® SGC	1.5 ml	
WEPRO®7240/7240H/7240G (240 OD)	0.5 ml	40 OD
Creatine Kinase (20 mg/ml)	6 µl	40 µg/ml
Total	3 ml	

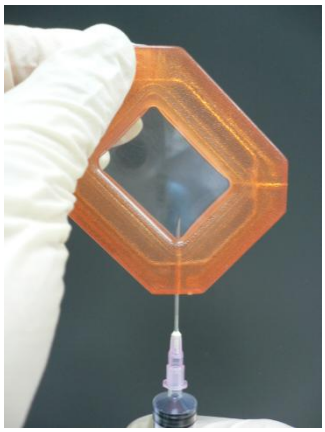
4.4 Prepare dialysis cassette

Before starting the experiment, make sure that the dialysis cassette in not damaged. Read the instructions provided by the maker, and note the positions of the syringe ports. Syringe ports should be used only once.

- Aspirate about 2 ml of nuclease-free water by syringe with needle.
- Insert the needle to the dialysis cassette and inject the water slowly from the top. Keep the needle inserted. Take care not to damage the dialysis membrane with the needle.



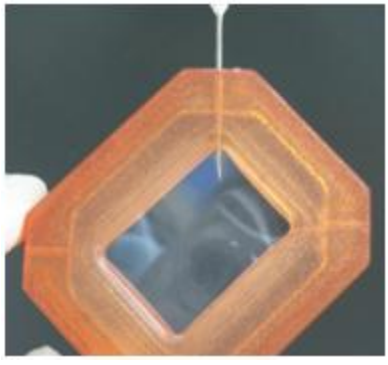
- Confirm the dialysis membrane does not leak the water.
- Invert the cassette to a position where the tip of the needle is facing up.



- Remove the water from the cassette using the syringe.
- Remove the needle slowly from the cassette.
- Soak the cassette in the SUB-AMIX® SGC buffer contained in the container for 2 min.
- Take out the cassette and wipe the rim of the cassette with a paper towel. Take care not to dry up the cassette.

4.5 Start dialysis reaction

- Aspirate the translation mixture by syringe with needle.
- Insert the needle to a new syringe port that has not been used before. Take care not to damage the dialysis membrane with the needle.



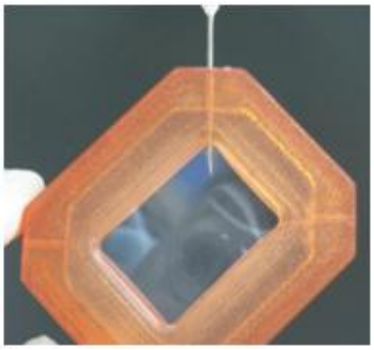
- Inject the translation mixture slowly. Keep the

needle inserted.

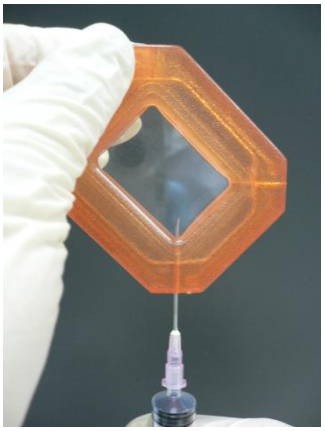
- After the injection, remove the air inside the cassette with the same syringe.
- Remove the needle slowly.
- Soak the cassette in the SUB-AMIX® SGC buffer contained in the container and cover the container.
- Shake the container by a shaker in an incubator at 15°C for up to 72 hours.

4.6 Collection of synthesized protein

- Take the dialysis cassette out from SUB-AMIX® SGC buffer and wipe the rim of the cassette with paper towel.
- Suck 3 ml of air with syringe with needle.
- Insert the needle to a syringe port that has not been used before and inject the air to the cassette slowly. Keep the needle inserted.



- Invert the cassette to a position where the tip of the needle is facing up.



- Collect the translation reaction with the syringe slowly.
- Remove the needle slowly.
- Transfer the solution into a tube.
- Take an aliquot of the reaction mixture for further analysis before starting protein use or purification.

5. BENCH NOTES

Print this bench note for setting up your experiments. Mark each step in the protocol after completion.

Setup transcription reaction:

Reagent	Volume	Final Concentration	Checkmark
Nuclease-free water	604 µl	-	
5x Transcription Buffer LM	210 µl	1x	
NTP Mix (25 mM)	105 µl	2.5 mM	
RNase Inhibitor (80 U/µl)	13.2 µl	1 U/µl	
SP6 RNA Polymerase (80 U/µl)	13.2 µl	1 U/µl	
Plasmid (circular DNA, 1 µg/µl)	105 µl	100 ng/µl	
Total	1,050 µl	INCUBATE 6 h at 37°C	

Setup translation buffer SUB-AMIX® SGC:

Reagent	Volume	Final Concentration	Checkmark
Nuclease-free water	112 ml		
40x SUB-AMIX® SGC (S1)	3.1 ml	1x	
40x SUB-AMIX® SGC (S2)	3.1 ml	1x	
40x SUB-AMIX® SGC (S3)	3.1 ml	1x	
40x SUB-AMIX® SGC (S4)	3.1 ml	1x	
Total	125 ml		

Setup translation reaction:

Reagent	Volume	Final Concentration	Checkmark
mRNA	1 ml	1/3 vol.	
1x SUB-AMIX® SGC	1.5 ml		
WEPRO®7240/7240H/7240G (240 OD)	0.5 ml	40 OD	
Creatine Kinase (20 mg/ml)	6 µl	40 µg/ml	
Total	3 ml	INCUBATE up to 72 h at 15°C	

- Make sure dialysis cassette is not damaged: Wash cassette with some 2 ml of water.
- Add reaction mix to dialysis cassette: Make sure to always use new syringe port on cassette.
- Place in feeding buffer and incubate at 15°C for up to 72 hours.
- Place dialysis reaction on shaker for better reagent exchange.
- Remove reaction mix from dialysis cassette using a new syringe port.

6. TROUBLESHOOTING

The experiments require correct and accurate pipetting during reaction setup. Any mistakes 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 number on each reagent tube prior to starting the pipetting step.

Mark in your protocol each pipetting step you have completed on the Bench Notes.

Change the pipetting tip after each pipetting step. Do not use the same pipetting tip to pipette different reagents or reaction mixtures.

Leaving out the plasmid template will always yield negative results.

Make sure not to damage the dialysis cassette.

If 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.

Contact the technical support of CellFree Sciences for further support and help.

7. LABEL LICENSE POLICY

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