ENDEXT® Technology

WEPRO7240/7240H/7240G Expression Kit

Instruction manual for protein synthesis
with wheat germ cell-free system

(Catalog No. CFS-TRI-7240, CFS-TRI-7240H, CFS-TRI-7240G)

CellFree Sciences Co., Ltd.

Table of Contents

1. Introduction 2

2. Protocol Overview 3

3. Materials 4
   3.1. Contents of the Kit 4
   3.2. Instruction for the Use of Reagents 4
   3.3. Materials to Be Prepared by the User 6
      3.3.1. Reagents for Plasmid DNA preparation 6
      3.3.2. Nuclease-free Water for Transcription and Translation 6

4. Protocols 7
   4.1. Plasmid DNA Construction 7
   4.2. Preparation of Plasmid DNA for Transcription 9
   4.3. Small Scale Protein Expression 10
      4.3.1. Transcription 10
      4.3.2. Translation 11
   4.4. Middle Scale Protein Expression 12
      4.4.1. Transcription 12
      4.4.2. Translation 13
   4.5. Large Scale Protein Expression 14
      4.5.1. Transcription 14
      4.5.2. Translation 15
   4.6. Detection of Target Protein by SDS-PAGE 16

5. Others 17
   5.1. Label License Policy 17
   5.2. Trademarks 17
   5.3. Others 17

6. Contact US 18

7. References 18

1. Introduction

WEPRO7240 Expression Kit

WEPRO7240 Expression Kit contains WEPRO®7240. It is suitable for protein expression where affinity purification to high purities is neither necessary nor desirable.

WEPRO7240H Expression Kit

WEPRO7240H Expression Kit is suitable for His-tagged protein expression and purification. The post-purification purity of the protein expressed with this kit is higher than that with WEPRO7240 Expression Kit. Please note that the yield of protein with this kit is slightly less than that with WEPRO7240 Expression Kit.

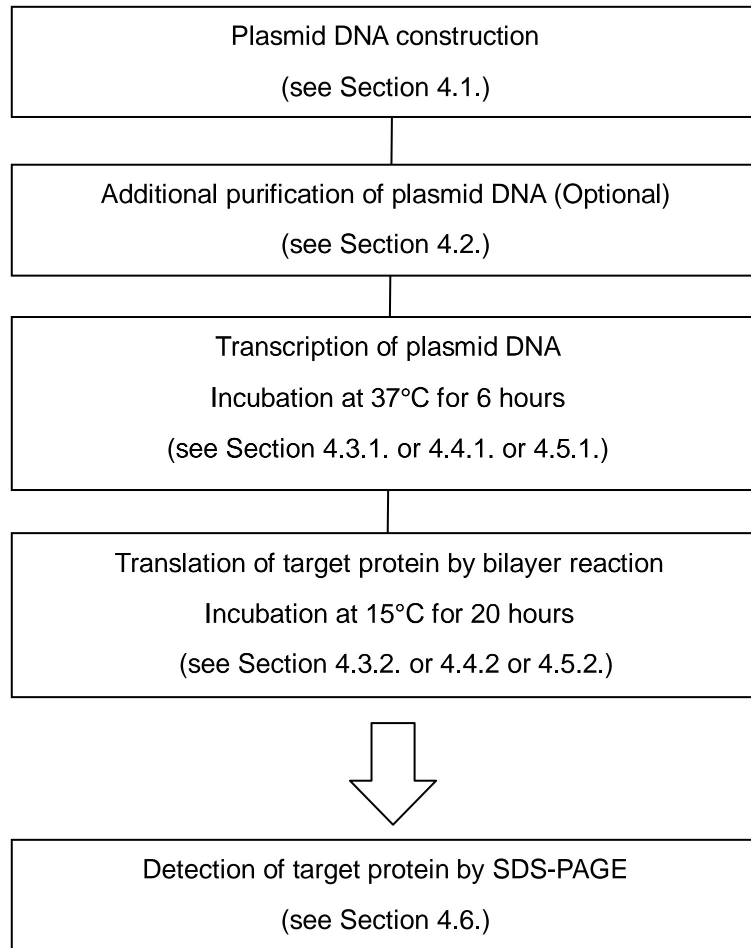
For the purification of His-tagged protein expressed using this kit, Ni-sepharose high performance resin of GE Healthcare is recommended. Please note that 1x SUB-AMIX® SGC and WEPRO®7240H include 4 mM dithiothreitol (DTT) and that some other resins are not resistant to DTT of this concentration. Please ensure that your resin is resistant to 4 mM DTT.

WEPRO7240G Expression Kit

WEPRO7240G Expression Kit is suitable for GST-tagged protein expression and purification. The post-purification purity of the protein expressed with this kit is higher than that with WEPRO7240 Expression Kit. Please note that the yield of protein with this kit is slightly less than that with WEPRO7240 Expression Kit.

For the purification of GST-tagged protein expressed using this kit, glutathione sepharose 4B resin of GE Healthcare is recommended. Please note that 1x SUB-AMIX® SGC and WEPRO®7240G include 4 mM dithiothreitol (DTT) and that some other resins are not resistant to DTT of this concentration. Please ensure that your resin is resistant to 4 mM DTT.

2. Protocol Overview



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3. Materials

3.1. Contents of the Kit

Item	Quantity	Concentration	Volume
WEPRO®7240/ WEPRO®7240H/ WEPRO®7240G	2	240 OD/ml	1 ml
SUB-AMIX® SGC S-1	1	40x	1.1 ml
SUB-AMIX® SGC S-2	1	40x	1.1 ml
SUB-AMIX® SGC S-3	1	40x	1.1 ml
SUB-AMIX® SGC S-4	1	40x	1.1 ml
5x Transcription Buffer LM	1	5x	0.4 ml
NTP Mix	1	25 mM	0.2 ml
SP6 RNA Polymerase	1	80 U/μl	30 μl
RNase Inhibitor	1	80 U/μl	30 μl
Creatine Kinase	1	20 mg/ml	20 μl
pEU-E01-MCS	1	1 μg/μl	10 μl
pEU-E01-DHFR	1	1 μg/μl	10 μl

3.2. Instruction for the Use of Reagents

Item	Description	Storage
WEPRO®7240/ WEPRO®7240H/ WEPRO®7240G	WEPRO®7240/7240H/7240G (wheat germ extract) is 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 volumes 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. Avoid bubbling.	-80°C
SUB-AMIX® SGC (S-1, S-2, S-3, S-4)	This product consists of a set of 4 reagents (S-1, S-2, S-3, S-4) 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 44 ml of 1x SUB-AMIX® SGC mixture, add 1.1 ml each of S-1 through S-4 to 39.6 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 exposures, subdivide 1x SUB-AMIX® SGC mixture into appropriate volumes 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

(Continued)

(Continued from Section 3.2.)

Item	Description	Storage
5x Transcription Buffer LM	After thawing, subdivide 5x Transcription Buffer LM into appropriate volumes 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	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 volumes 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	50% glycerol is included.	-20°C
RNase Inhibitor	50% glycerol is included.	-20°C
Creatine Kinase (*1)	Creatine Kinase is required for protein expression. Avoid multiple freeze-thawing exposures; otherwise, the activity of Creatine Kinase may decrease. The concentration of the starting Creatine Kinase solution is 20 mg/ml. Use it as it is for preparing the translation mixture for large scale and middle scale translation. For small scale translation (see Section 4.3.2.), dilute it with nuclease-free water to 1 mg/ml. In both cases, the final concentration of Creatine Kinase in translation mixture should be 40 ng/µl.	-80°C
pEU-E01-MCS	Expression vector for subcloning your gene of interest. See section 4.1. in detail.	-20°C
pEU-E01-DHFR	Expression vector encoding dihydrofolate reductase (DHFR) gene derived from *E. coli*. It works as positive control for protein expression. It has no tag sequence such as GST or His.	-20°C

(Notes)

*1 Creatine Kinase can be purchased from Roche Applied Science, Catalog No. 10127566001. Dissolve it with nuclease-free water to make a 20 mg/ml solution. For convenience, subdivide the solution into smaller volumes and store them at -80°C. Avoid multiple freeze-thawing cycles; otherwise, the activity of Creatine Kinase may decrease.

3.3. Materials to Be Prepared by the User

3.3.1. Reagents for Plasmid Preparation

The following reagents are necessary to prepare plasmid DNA for transcription (see Section 4.1 and 4.2).

Reagents	Description
Phenol/Chloroform	phenol:chloroform:isoamyl alcohol = 25:24:1 in volume, pH 7.9
Chloroform	> 99%
Ethanol	2 grades: > 99% and 70 %
Sodium acetate	3 M, pH 5.2
TE buffer	10 mM Tris, 1 mM EDTA, pH 8.0. Sterilized. It is highly recommended to use DNase-RNase free water when you prepare TE buffer. We DO NOT recommend homemade DEPC treated water.

3.3.2. Nuclease-free Water for Transcription and Translation

Nuclease-free water is necessary to prepare the reagents for transcription and translation.

Reagents	Description
Nuclease-free water	DNase, RNase free. We DO NOT recommend homemade DEPC treated water.

4. Protocols

4.1. Plasmid DNA Construction

1) Insert your cDNA into the multiple cloning site (MCS) of the pEU-E01-MCS vector with restriction enzymes properly selected according to the MCS information given on next page (*1, *2). Protein is translated from the first start codon ATG to stop codon in your cDNA inserted in the MCS. Please note that pEU-E01-MCS contains SP6 promoter, E01 translational enhancer, and ampicillin resistance gene as illustrated on next page.
2) Cultivate *E. coli* containing the cDNA-inserted pEU-E01-MCS.
3) Extract the plasmid DNA from *E. coli* and purify it with a commercially available kit, for example, one from QIAGEN. We recommend QIAGEN Plasmid Midi Kit (catalog No. 12143) or QIAGEN Plasmid Maxi Kit (catalog No. 12163). We DO NOT recommend mini-prep method for the present purpose.
4) After the purification, determine the concentration of the DNA with a spectrophotometer at the wavelengths of 260 nm and 280 nm. Absorbance at 260 nm represents the concentration of DNA. The ratio of absorbance at 260 nm to that at 280 nm represents the purity of the DNA (*3).
5) Adjust the DNA concentration to 1.0 µg/µl by adding an appropriate volume of TE buffer.

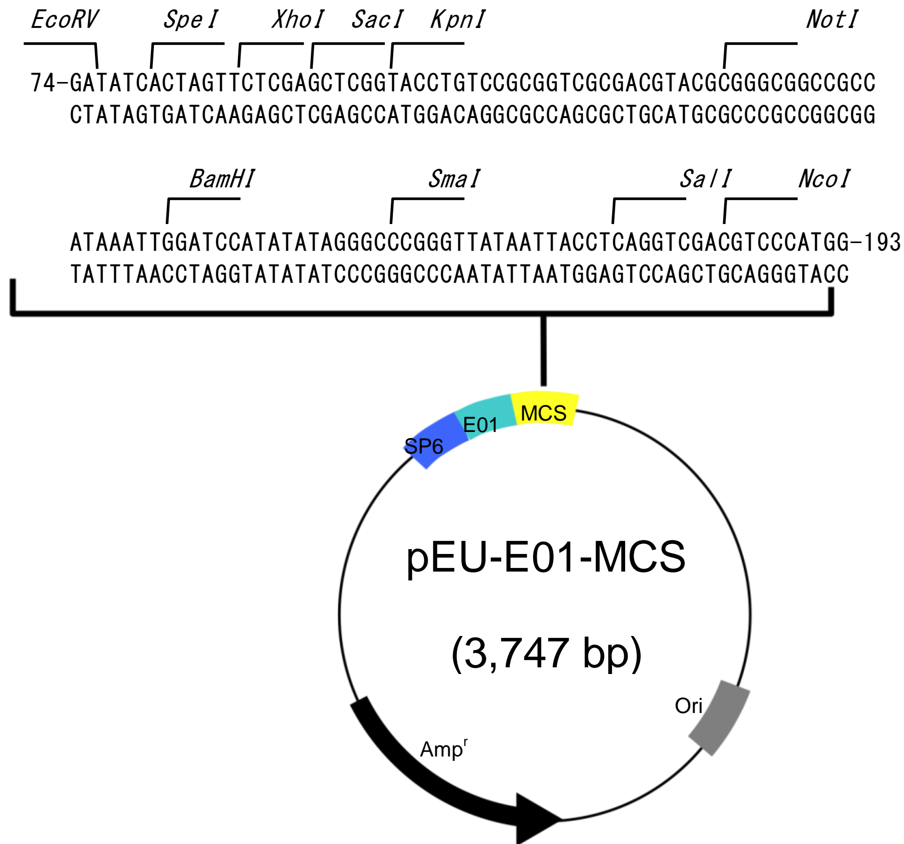
(Notes)

*1 In order to efficiently express the target protein, it is recommended to select a restriction enzyme site as close as possible to E01 translational enhancer.

*2 It is NOT recommended to select *Xho* I restriction enzyme site alone, because self-ligation may occur. Should you use *Xho* I site, use *Sal* I site in combination with *Xho* I site.

*3 Purity of plasmid DNA should be such that the A260/A280 ratio ranges between 1.70 and 1.85. Ratios outside the range indicate that the plasmid DNA has been contaminated. In that case, further purify the plasmid DNA as described in Section 4.2.

(Multiple cloning site information)



pEU-E01-MCS sequence

SP6 promoter: -17~1
Translational enhancer (E01): 16~72
Multiple Cloning Site: 74~193
Origin: 1190~1830
Ampicillin resistance gene: 1974~2838

Position 1 is located at the final G
(underlined in the following sequence)
of SP6 promoter: ATTTAGGTGACACTATA<u>G</u>

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4.2. Preparation of Plasmid DNA for Transcription

A high purity plasmid DNA is essential for successful transcription and subsequent translation. If the plasmid DNA purified with a commercially available kit is contaminated or the quality of transcripts made with the plasmid DNA is low, protein synthesis may not be successful. In that case, further purification of the plasmid DNA may be necessary.

This additional purification, which is optional if plasmid DNA has been prepared properly, is accomplished by extraction first with phenol/chloroform and then with chloroform, and by precipitation with ethanol as described below:

1) Add an equal volume of phenol/chloroform (phenol:chloroform:isoamyl alcohol = 25:24:1, pH 7.9) to the purified plasmid DNA solution (see Section 4.1.) and mix well.
2) Centrifuge the mixture at 15,000 rpm for 5 min.
3) Carefully transfer the upper aqueous phase to a new tube.
4) Add an equal volume of chloroform into the tube and mix well.
5) Centrifuge this mixture at 15,000 rpm for 5 min.
6) Carefully transfer the upper aqueous phase to another new tube.
7) To this upper aqueous solution, add 100% ethanol, 2.5 times the volume, and 3M sodium acetate (pH 5.2), 1/10 of the volume, to precipitate the DNA.
8) Hold at -20°C for 10 min.
9) Centrifuge at 15,000 rpm for 20 min at 4°C.
10) Remove the supernatant. Add 800 µl of 70% ethanol to wash the remaining DNA pellet in the tube.
11) Centrifuge the tube at 15,000 rpm for 10 min at 4°C.
12) Remove the supernatant.
13) Dry the DNA pellet for 10 to 20 min.
14) Add an appropriate volume of TE buffer to resuspend the DNA pellet.
15) Determine the concentration of the DNA with a spectrophotometer at the wavelengths of 260 nm and 280 nm. Absorbance at 260 nm represents the concentration of DNA. The ratio of absorbance at 260 nm to that at 280 nm represents the purity of the DNA (*1).
16) Adjust the DNA concentration to 1.0 µg/µl by adding an appropriate volume of TE buffer.

(Notes)

*1 Purity of plasmid DNA should be such that the A260/A280 ratio ranges between 1.70 and 1.85. Ratios outside the range indicate that the plasmid DNA has been contaminated. In that case, repeat Section 4.2 from the beginning.

4.3. Small Scale Protein Expression

The following description is for small scale protein expression using a standard 96 multi-well plate. It is for a translation reaction volume of 226.8 µl per well.

4.3.1. Transcription

1) Thaw 5x Transcription Buffer LM and NTP Mix on ice. After thawing, spin down the tubes for a short time to drop down the reagent staying on the tube wall or on the cap. To avoid localized concentration, mix the reagent gently before using it. Place and keep all reagents on ice during handling.
2) Prepare 20 µl of transcription mixture on ice according to the mixing formula shown below and mix gently by pipetting.

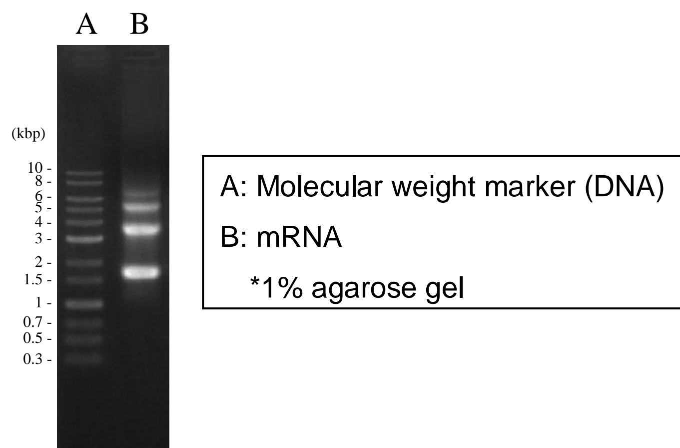
Reagents	Working vol.	Final conc.
Nuclease-free water	11.5 µl	-
5x Transcription Buffer LM	4 µl	1x
NTP Mix (25 mM)	2 µl	2.5 mM
RNase Inhibitor (80 U/µl)	0.25 µl	1 U/µl
SP6 RNA Polymerase (80 U/µl)	0.25 µl	1 U/µl
Plasmid (circular DNA, 1 µg/µl)	2 µl	100 ng/µl
Total	20 µl	

3) Incubate at 37°C for 6 hours in a thermal cycler or incubator (*1).
4) After incubation, confirm the mRNA quality by the ordinary method of agarose gel electrophoresis (*2).

(Notes)

*1 White precipitate may appear during the incubation. This is magnesium pyrophosphate. Use the whole mixture including precipitate in the next step.

*2 A smear or ladder pattern of less than 500 bases of mRNA indicates possible degradation of mRNA probably caused by RNase. In that case, further purify the plasmid DNA as described in Section 4.2. An example of mRNA produced in high quality is shown below:



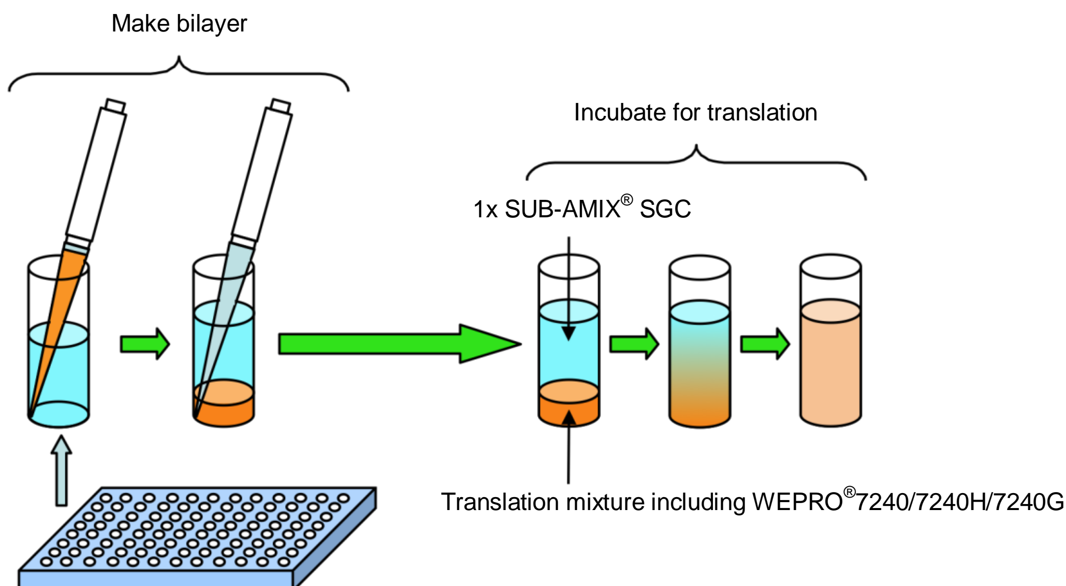
4.3.2. Translation

1) Let the mRNA tube cool down to the room temperature. DO NOT forcibly cool it on ice or in the refrigerator. Before adding mRNA into the translation mixture, resuspend the white pellet (magnesium pyrophosphate) in the tube by pipetting gently.
2) Thaw WEPRO®7240/7240H/7240G under running water, and immediately after thawing, place it on ice. Thaw Creatine Kinase on ice. After thawing the reagents, spin down the tubes for a short time to drop down the reagent staying on the tube wall or on the cap. Avoid excessive centrifugation. To avoid localized concentration, mix the reagent gently before using it. Avoid bubbling.
3) Prepare 20.8 µl of translation mixture on ice according to the mixing formula shown below and mix gently by pipetting. Avoid bubbling.

Reagents	Working vol.	Final conc.
mRNA	10 µl	1/2 vol.
Creatine Kinase (1 mg/ml)	0.8 µl	40 ng/µl
WEPRO®7240/7240H/7240G (240 OD/ml)	10 µl	120 OD/ml
Total	20.8 µl	

4) Thaw 1x SUB-AMIX® SGC on ice and mix gently by pipetting. Pipet out 206 µl of 1x SUB-AMIX® SGC and add it into a well of a 96 multi-well, flat bottom plate.
5) **Carry out bilayer reaction: Carefully transfer the translation mixture into the bottom of the well containing 1x SUB-AMIX® SGC to form bilayer with the translation mixture in the lower layer and 1x SUB-AMIX® SGC in the upper layer as illustrated below. DO NOT mix the reagents in the well by pipetting or any other means. (Important !!)**
6) Seal the well with Parafilm to avoid evaporation.
7) Incubate at 15˚C for 20 hours.
8) After translation, mix the bilayer reaction gently by pipetting for later experimental usage.

Bilayer reaction system



4.4. Middle Scale Protein Expression

The following description is for middle scale protein expression using a standard 24 multi-well plate. It is for a translation reaction volume of 1.2 ml per well.

4.4.1. Transcription

1) Thaw 5x Transcription Buffer LM and NTP Mix on ice. After thawing, spin down the tubes for a short time to drop down the reagent staying on the tube wall or on the cap. To avoid localized concentration, mix the reagent gently before using it. Place and keep all reagents on ice during handling.
2) Prepare 50 µl of transcription mixture on ice according to the mixing formula shown below and mix gently by pipetting.

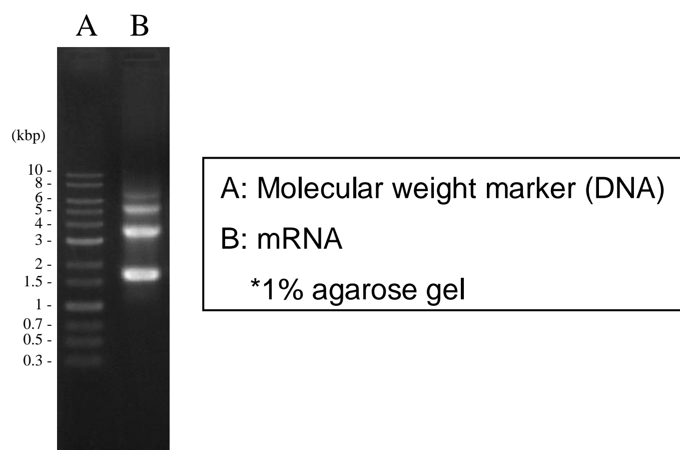
Reagents	Working vol.	Final conc.
Nuclease-free water	28.75 µl	-
5x Transcription Buffer LM	10 µl	1x
NTP Mix (25 mM)	5 µl	2.5 mM
RNase Inhibitor (80 U/µl)	0.625 µl	1 U/µl
SP6 RNA Polymerase (80 U/µl)	0.625 µl	1 U/µl
Plasmid (circular DNA, 1 µg/µl)	5 µl	100 ng/µl
Total	50 µl	

3) Incubate at 37°C for 6 hours in a thermal cycler or incubator (*1).
4) After incubation, confirm the mRNA quality by the ordinary method of agarose gel electrophoresis (*2).

(Notes)

*1 White precipitate may appear during the incubation. This is magnesium pyrophosphate. Use the whole mixture including precipitate in the next step.

*2 A smear or ladder pattern of less than 500 bases of mRNA indicates possible degradation of mRNA probably caused by RNase. In that case, further purify the plasmid DNA as described in Section 4.2. An example of mRNA produced in high quality is shown below:



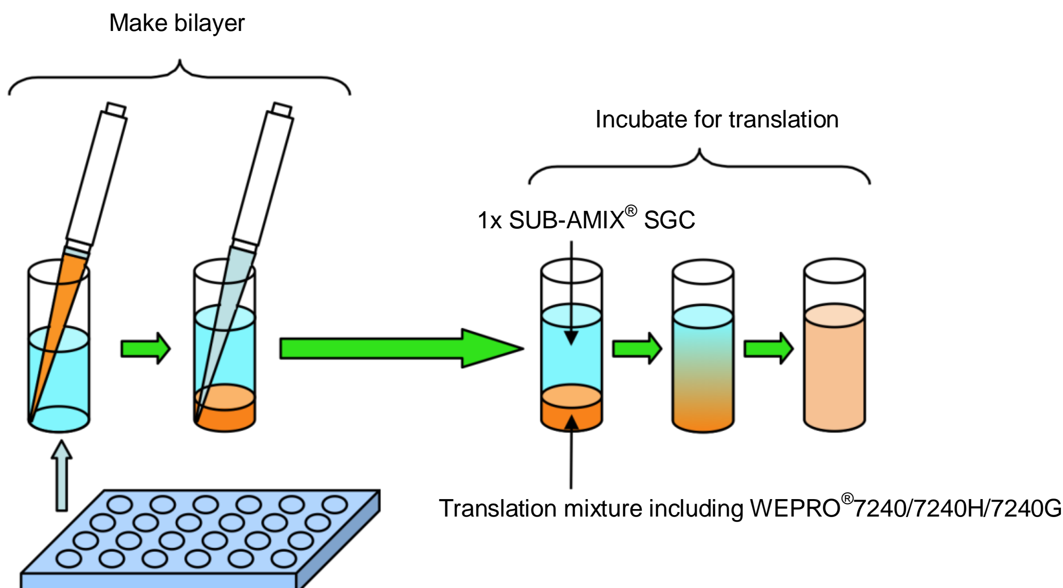
4.4.2. Translation

1) Let the mRNA tube cool down to the room temperature. DO NOT forcibly cool it on ice or in the refrigerator. Before adding mRNA into the translation mixture, resuspend the white pellet (magnesium pyrophosphate) in the tube by pipetting gently.
2) Thaw WEPRO®7240/7240H/7240G under running water, and immediately after thawing, place it on ice. Thaw Creatine Kinase on ice. After thawing the reagents, spin down the tubes for a short time to drop down the reagent staying on the tube wall or on the cap. Avoid excessive centrifugation. To avoid localized concentration, mix the reagent gently before using it. Avoid bubbling.
3) Prepare 100.2 µl of translation mixture on ice according to the mixing formula shown below and mix gently by pipetting. Avoid bubbling.

Reagents	Working vol.	Final conc.
mRNA	50 µl	1/2 vol.
Creatine Kinase (20 mg/ml)	0.2 µl	40 ng/µl
WEPRO®7240/7240H/7240G (240 OD/ml)	50 µl	120 OD/ml
Total	100.2 µl	

4) Thaw 1x SUB-AMIX® SGC on ice and mix gently by pipetting. Pipet out 1.1 ml of 1x SUB-AMIX® SGC and add it into a well of a 24 multi-well, flat bottom plate.
5) **Carry out bilayer reaction: Carefully transfer the translation mixture into the bottom of the well containing 1x SUB-AMIX® SGC to form bilayer with the translation mixture in the lower layer and 1x SUB-AMIX® SGC in the upper layer as illustrated below. DO NOT mix the reagents in the well by pipetting or any other means. (Important !!)**
6) Seal the well with Parafilm to avoid evaporation.
7) Incubate at 15˚C for 20 hours.
8) After translation, mix the bilayer reaction gently by pipetting for later experimental usage.

Bilayer reaction system



4.5. Large Scale Protein Expression

The following description is for large scale protein expression using a standard 6 multi-well plate. It is for a translation reaction volume of 6 ml per well.

4.5.1. Transcription

1) Thaw 5x Transcription Buffer LM and NTP Mix on ice. After thawing, spin down the tubes for a short time to drop down the reagent staying on the tube wall or on the cap. To avoid localized concentration, mix the reagent gently before using it. Place and keep all reagents on ice during handling.
2) Prepare 250 µl of transcription mixture on ice according to the mixing formula shown below and mix gently by pipetting.

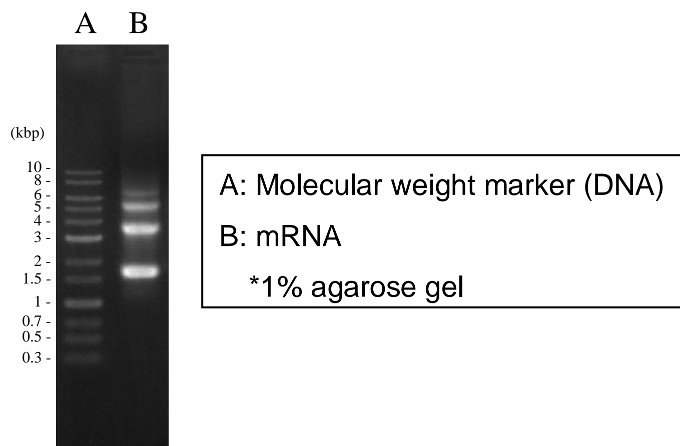
Reagents	Working vol.	Final conc.
Nuclease-free water	143.75 µl	-
5x Transcription Buffer LM	50 µl	1x
NTP Mix (25 mM)	25 µl	2.5 mM
RNase Inhibitor (80 U/µl)	3.125 µl	1 U/µl
SP6 RNA Polymerase (80 U/µl)	3.125 µl	1 U/µl
Plasmid (circular DNA, 1 µg/µl)	25 µl	100 ng/µl
Total	250 µl	

3) Incubate at 37°C for 6 hours in a thermal cycler or incubator (*1).
4) After incubation, confirm the mRNA quality by the ordinary method of agarose gel electrophoresis (*2).

(Notes)

*1 White precipitate may appear during the incubation. This is magnesium pyrophosphate. Use the whole mixture including precipitate in the next step.

*2 A smear or ladder pattern of less than 500 bases of mRNA indicates possible degradation of mRNA probably caused by RNase. In that case, further purify the plasmid DNA as described in Section 4.2. An example of mRNA produced in high quality is shown below:



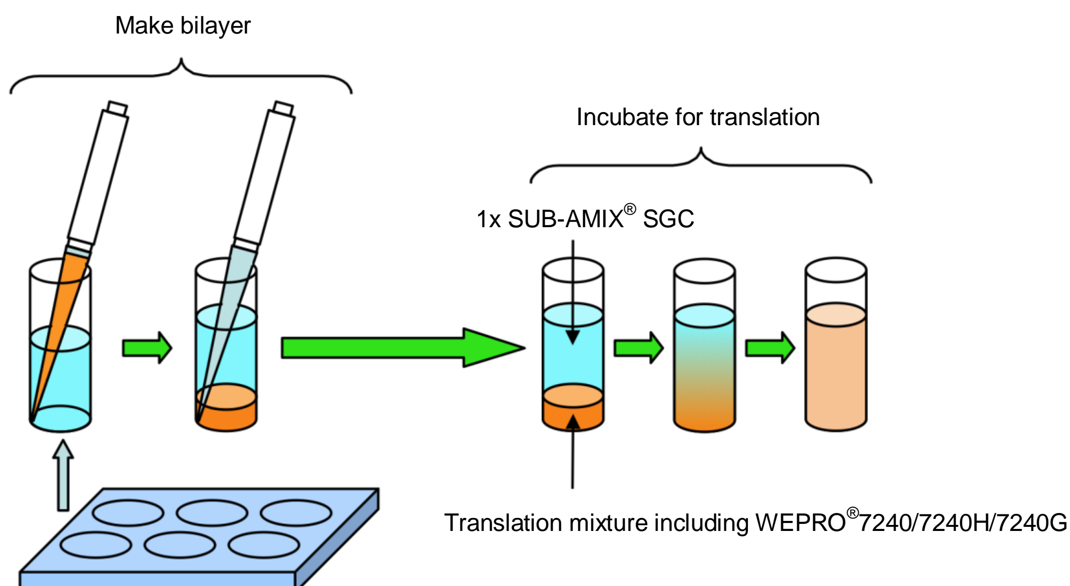
4.5.2. Translation

1) Let the mRNA tube cool down to the room temperature. DO NOT forcibly cool it on ice or in the refrigerator. Before adding mRNA into the translation mixture, resuspend the white pellet (magnesium pyrophosphate) in the tube by pipetting gently.
2) Thaw WEPRO®7240/7240H/7240G under running water, and immediately after thawing, place it on ice. Thaw Creatine Kinase on ice. After thawing the reagents, spin down the tubes for a short time to drop down the reagent staying on the tube wall or on the cap. Avoid excessive centrifugation. To avoid localized concentration, mix the reagent gently before using it. Avoid bubbling.
3) Prepare 501 µl of translation mixture on ice according to the mixing formula shown below and mix gently by pipetting. Avoid bubbling.

Reagents	Working vol.	Final conc.
mRNA	250 µl	1/2 vol.
Creatine Kinase (20 mg/ml)	1 µl	40 ng/µl
WEPRO®7240/7240H/7240G (240 OD/ml)	250 µl	120 OD/ml
Total	501 µl	

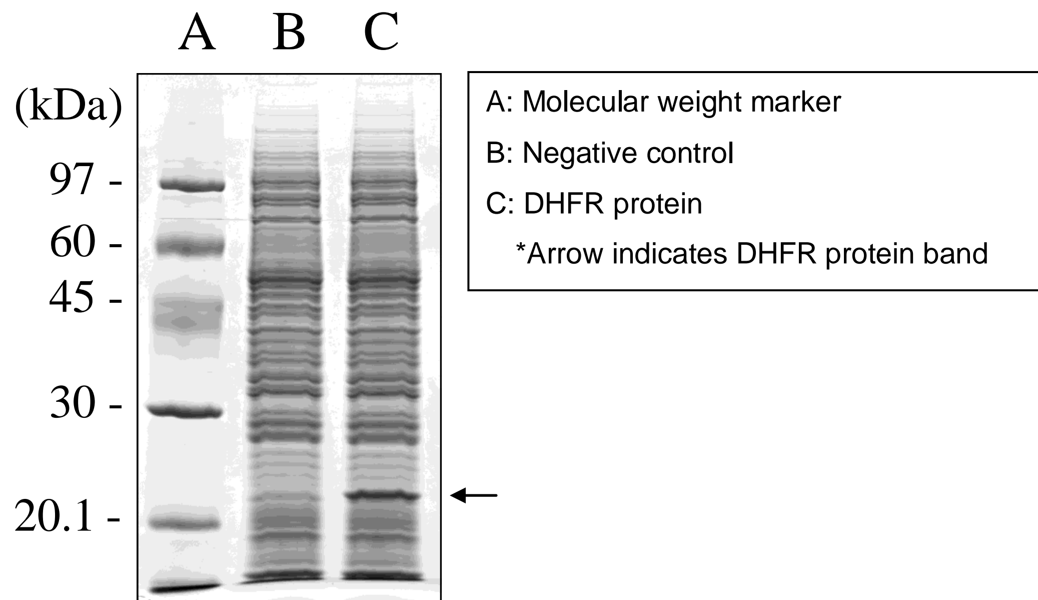
4) Thaw 1x SUB-AMIX® SGC on ice and mix gently by pipetting. Pipet out 5.5 ml of 1x SUB-AMIX® SGC and add it into a well of a 6 multi-well, flat bottom plate.
5) **Carry out bilayer reaction: Carefully transfer the translation mixture into the bottom of the well containing 1x SUB-AMIX® SGC to form bilayer with the translation mixture in the lower layer and 1x SUB-AMIX® SGC in the upper layer as illustrated below. DO NOT mix the reagents in the well by pipetting or any other means. (Important !!)**
6) Seal the well with Parafilm to avoid evaporation.
7) Incubate at 15˚C for 20 hours.
8) After translation, mix the bilayer reaction gently by pipetting for later experimental usage.

Bilayer reaction system



4.6. Detection of Target Protein by SDS-PAGE

Run SDS-PAGE followed by CBB staining to identify the expressed protein. SDS-PAGE requires high resolution and an appropriate gel concentration to distinguish the expressed protein from background proteins originating from wheat germ. Load 3 µl of sample for SDS-PAGE. If the volume is too high or too low to identify the protein, change the volume to obtain a clear result. DHFR protein used as positive control is expressed as approx. 20 kDa protein. Typical CBB-stained gel data is shown below.



5. Others

5.1. Label License Policy

By opening the cap of any of the reagents listed in the above Section 3.1, the buyer of the WEPRO7240/7240H/7240G Expression Kit is agreeing to be bound by the terms of the following Label License Policy.

<< Label License Policy>>
ENDEXT® technology and products are covered by US Patent Nos. 6905843, 6869774 and 7919597, 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.

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5.3. Others

All specifications are subject to change without prior notice.

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Web site: http://www.cfsciences.com

7. References

Y. Endo and T. Sawasaki (2004). High-throughput, genome-scale protein production method based on the wheat germ cell-free expression system. *J. Struct. Funct. Genomics* **5**, 45-57.

T. Sawasaki, T. Ogasawara, R. Morishita and Y. Endo (2002). A cell-free protein synthesis system for high-throughput proteomics. *Proc. Natl. Acad. Sci. USA* **99**, 14652-14657.

T. Sawasaki, Y. Hasegawa, M. Tsuchimochi, N. Kamura, T. Ogasawara and Y. Endo (2002). A bilayer cell-free protein synthesis system for high-throughput screening of gene products. *FEBS Lett.* **514**, 102-105.

K. Madin, T. Sawasaki, T. Ogasawara and Y. Endo (2000). A highly efficient and robust cell-free protein synthesis system prepared from wheat embryos. *Proc. Natl. Acad. Sci. USA* **97**, 559-564.

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