Automated Protein Synthesizer

Protemist DT II

Reagent Preparation Manual

CellFree Sciences Co., Ltd.

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1. Introduction

Protemist® DT II synthesizes protein of interest using ENDEXT® wheat germ cell-free system and through bi-layer reaction. It can run two scales of translation reaction, either 1.2 ml or 6 ml. Batch affinity purification is available to purify GST- or 6x histidine-tagged fusion proteins synthesized in 6-ml translation scale.

This manual concerns the reagents and their preparation required for protein synthesis and purification using Protemist® DT II. It covers the following reactions.

- 1.2-ml translation scale
- 6-ml translation scale
- 6-ml translation scale of GST-tagged protein and its purification using reduced glutathione buffer for elution
- 6-ml translation scale of GST-tagged protein and its purification using AcTEV™ protease for elution
- 6-ml translation scale of GST-tagged protein and its purification using PreScission protease for elution
- 6-ml translation scale of His-tagged protein and its purification using imidazole buffer for elution
- 6-ml translation scale of His-tagged protein and its purification using AcTEV™ protease for elution

Note: Tag cleavage efficiency of a protease in elution is highly variable and affected by the nature of the protein.

For more information on the Protemist® DT II, please refer to the Instruction Manual.

2. Materials to be Prepared by the User

2-1. Off-the-shelf reagents

For plasmid DNA preparation

Reagents	Description	Storage
Nuclease-free water	DNase, RNase free. We DO NOT recommend homemade DPEC treated water.	4℃
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 DPEC treated water.	4℃

For transcription and translation

Reagents	Description	Storage
Nuclease-free water	DNase, RNase free. We DO NOT recommend homemade DPEC treated water.	4℃

For translation

Reagents	Description	Storage
Creatine Kinase	Creatine Kinase can be purchased from Sigma-Aldrich, 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.	-80°C

2-2. Reagents available from CellFree Sciences for transcription and translation

Item	Description	Storage
5x Transcription Buffer/ 5x Transcription Buffer LM	After thawing, subdivide 5x Transcription Buffer/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
25 mM 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
RNase Inhibitor (80,000 unit/ml)	50% glycerol is included.	-20°C
SP6 RNA Polymerase (80,000 unit/ml)	50% glycerol is included.	-20°C
WEPRO®1240 */ WEPRO®7240 *	WEPRO®1240/WEPRO®7240 (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® (S-1, S-2, S-3, S-4)/ 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. It is convenient to divide the 40x concentrated reagents into 1 ml after thawing them for the first time, and then store them at -20℃ or below. To prepare 40 ml of 1x SUB-AMIX®/SUB-AMIX® SGC, add 1 ml each of S-1 through S-4 to 36 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®/SUB-AMIX® SGC into appropriate volumes in separate containers and store them at -80°C. Do not subject 1x SUB-AMIX®/SUB-AMIX® SGC 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

*: To improve the purity of GST-tagged and His-tagged proteins, we recommend WEPRO®1240G and WEPRO®1240H, respectively.

2-3. Reagents combination for transcription and translation

Use WEPRO®1240 and WEPRO®7240 in combination with those reagents marked by ○ in the table below:

WEPRO®1240	WEPRO®7240	Reagents
○		5x Transcription Buffer
	○	5x Transcription Buffer LM
○	○	25 mM NTP Mix
○	○	RNase Inhibitor
○	○	SP6 RNA Polymerase
○		SUB-AMIX®
	○	SUB-AMIX® SGC

2-4. Reagents for protein purification

Select and prepare proper reagents according to the tag and purification method of the protein of interest.

GST-tag			His-tag		Reagents
Reduced glutathione	AcTEV™ protease	PreScission protease	Imidazole	AcTEV™ protease	
○	○	○			GST resin
			○	○	His resin
○	○	○			Wash buffer A
			○	○	Wash buffer B
			○	○	Solution A
○					Elution buffer A
	○				Elution buffer B
		○			Elution buffer C
	○	○			Elution buffer D
			○		Elution buffer E
				○	Elution buffer F
				○	Elution buffer G

Reagents	Description	Storage
GST resin	Glutathione sepharose 4B of Cytiva. Replace the storage solution with wash buffer A to prepare 50% slurry (*1).	4℃ (*1)
His resin	Ni Sepharose 6 Fast Flow of Cytiva. Replace the storage solution with wash buffer B to prepare 50% slurry (*1).	4℃ (*1)
Wash buffer A	Phosphate buffered saline	4℃ (*2)
Wash buffer B	20 mM Na-phosphate pH 7.5, 0.3 M NaCl, 20 mM imidazole	4℃ (*2)
Solution A	600 mM imidazole pH 8.0	4℃
Elution buffer A	50 mM Tris-HCl, 10 mM reduced glutathione, pH 8.0	- 20℃
Elution buffer B	1 unit/µl (final conc.) of AcTEV™ protease (Thermo Fisher Scientific) in phosphate buffered saline (*1)	*3
Elution buffer C	0.08 unit/µl (final conc.) of PreScission protease (Cytiva) in phosphate buffered saline (*1)	*3
Elution buffer D	Phosphate buffered saline	4℃ (*2)
Elution buffer E	20 mM Na-phosphate pH 7.5, 0.3 M NaCl, 500 mM imidazole	4℃ (*2)
Elution buffer F	1 unit/µl (final conc.) of AcTEV™ protease (Thermo Fisher Scientific) in elution buffer G (*1)	*3
Elution buffer G	20 mM Na-phosphate pH 7.5, 0.3 M NaCl	4℃ (*2)

*1: See manufacture's instruction manual for details.

*2: Long-term storage is not recommended.

*3: Prepare just before use.

2-5. Reagent for cleaning accessories

Reagents	Description
RNase decontaminant	e.g. RNase AWAY, Molecular BioProducts, Catalog No. 7002.

3. Preparation of Plasmid DNA

For the purification of GST- or His-tagged proteins, add the GST- or His-tag sequence to your gene of interest. If the protein is to be eluted by cleaving the tag with a protease, i.e. AcTEV™ or PreScission, add the protease cleavage site between the tag and your gene of interest.

3-1. Plasmid DNA construction

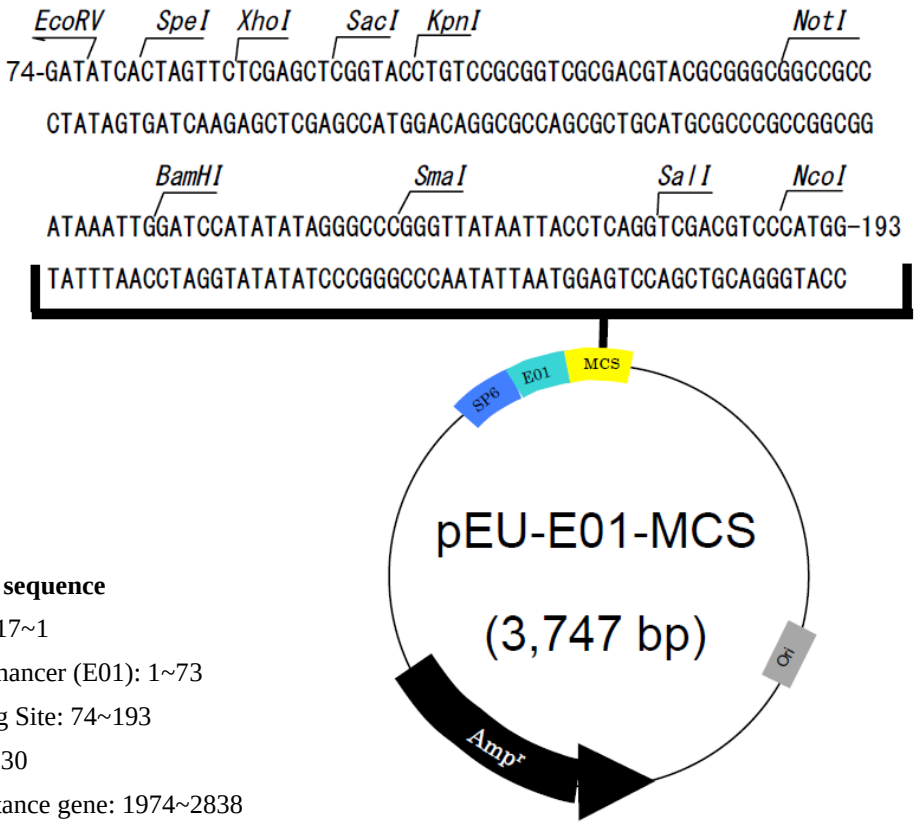
1. Insert your cDNA into the multiple cloning site (MCS) of the vector "pEU-E01-MCS" with restriction enzymes properly selected according to the MCS information given on next page (*1, *2). Protein is translated from the first start codon “ATG” to stop codon in your cDNA inserted in the MCS. Please note that the 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, the 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 this 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.

Note*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.

Note*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.

Note*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 3-2.

(Multiple cloning site information)



pEU-E01-MCS sequence

SP6 promoter: -17~1

Translational enhancer (E01): 1~73

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: ATTTAGGTGACACTATAG

3-2. Additional purification of plasmid DNA (optional)

A highly purified plasmid DNA is required for the transcription and subsequent translation. In the case of case of the plasmid DNA purified with commercially available kit is contaminated or the transcripts quality of the plasmid DNA is low, the protein synthesis may not be carried out properly. Do further purification of the plasmid DNA when the protein is not synthesized. As described below, this additional purification is accomplished by extraction first with phenol/chloroform and then with chloroform, and by ethanol precipitation:

1. To the purified plasmid DNA solution, add an equal volume of phenol/chloroform (phenol:chloroform:isoamyl alcohol = 25:24:1, pH 7.9) 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 of the solution, and 3 M 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.

Note *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 3-2 from the beginning.

4. Preparation of the Reagents

Note: All reagents should be prepared on ice. Treat the reagents with hand gloves to avoid RNase contamination.

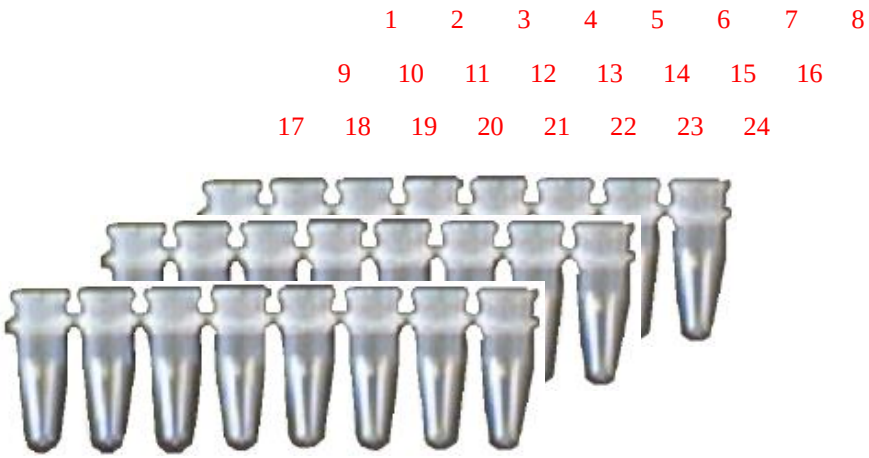
Use WEPRO®1240 and WEPRO®7240 in combination with those reagents marked by ○ in the table below:

WEPRO®1240	WEPRO®7240	Reagents
○		5x Transcription Buffer
	○	5x Transcription Buffer LM
○	○	25 mM NTP Mix
○	○	RNase Inhibitor
○	○	SP6 RNA Polymerase
○		SUB-AMIX®
	○	SUB-AMIX® SGC

4-1. Reagents for 1.2-ml translation scale

Plasmid DNA

Place 5 µl of pEU-E01 plasmid vector (1 µg/µl) containing your gene of interest in a 0.2-ml strip microtube from left to right as indicated below:



Transcription mixture

Thaw 5x Transcription Buffer/5x Transcription Buffer LM and 25 mM 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.

Prepare the subscribed volume of reagent mixture taking into account the dead volume for each reagent shown in the table below:

[required volume] = [volume of 1 sample] x [number of sample] + [dead volume]

Mix the reagents in a 2-ml microtube.

Reagents	1 sample	Dead volume	Final conc.
Nuclease-free water	28.75 µl	86.25 µl	
5x Transcription Buffer/ 5x Transcription Buffer LM	10 µl	30 µl	1x
25 mM NTP Mix	5 µl	15 µl	2.5 mM
SP6 RNA Polymerase (80,000 unit/ml)	0.625 µl	1.875 µl	1 unit/µl
RNase Inhibitor (80,000 unit/ml)	0.625 µl	1.875 µl	1 unit/µl
Total	**45 µl**	**135 µl**	

Translation mixture

Thaw WEPRO®1240/WEPRO®7240 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.

Prepare the subscribed volume of reagent mixture taking into account the dead volume for each reagent shown in the table below:

[required volume] = [volume of 1 sample] x [number of sample] + [dead volume]

Mix the reagents and place them in a 2-ml microtube (*).

Reagents	1 sample	Dead volume	Final conc.
WEPRO®1240/ WEPRO®7240	50 µl	175 µl	240 OD
Creatine Kinase (20 µg/µl)	0.2 µl	0.7 µl	80 ng/µl
Total	**50.2 µl**	**175.7 µl**	

Note (*): If bubbling occurs, ensure that the central area of the surface of the mixture in the 2-ml tube is free of bubbles.

Translation buffer

Thaw 1x SUB-AMIX®/1x SUB-AMIX® SGC on ice and mix gently by pipetting.

Prepare the subscribed volume of reagent mixture taking into account the dead volume for each reagent shown in the table below:

[required volume] = [volume of 1 sample] x [number of sample] + [dead volume]

Place the reagent in a 50-ml tube.

Reagents	1 sample	Dead volume
1x SUB-AMIX®/ 1x SUB-AMIX® SGC	1.1 ml	1.5 ml

4-2. Reagents for 6-ml translation scale

Prepare subscribed volumes of reagent mixtures according to the number of samples as shown in each table below. Dead volumes are included in the subscribed volumes.

Use WEPRO®1240 and WEPRO®7240 in combination with those reagents marked by ○ in the table below:

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WEPRO®1240	WEPRO®7240	Reagents
○		5x Transcription Buffer
	○	5x Transcription Buffer LM
○	○	25 mM NTP Mix
○	○	RNase Inhibitor
○	○	SP6 RNA Polymerase
○		SUB-AMIX®
	○	SUB-AMIX® SGC

Plasmid DNA

Place 25 µl of pEU-E01 plasmid vector (1 µg/µl) containing your gene of interest in a 2-ml microtube.

Transcription mixture

Thaw 5x Transcription Buffer/5x Transcription Buffer LM and 25 mM 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.

Mix the reagents in a 2 ml microtube.

Reagents	Number of samples						Final conc.
	1	2	3	4	5	6	
Nuclease-free water	230 µl	374 µl	518 µl	661 µl	805 µl	949 µl	
5x Transcription Buffer/ 5x Transcription Buffer LM	80 µl	130 µl	180 µl	230 µl	280 µl	330 µl	1x
25 mM NTP Mix	40 µl	65 µl	90 µl	115 µl	140 µl	165 µl	2.5 mM
SP6 RNA Polymerase (80,000 unit/ml)	5 µl	8.1 µl	11.3 µl	14.4 µl	17.5 µl	20.6 µl	1 unit/µl
RNase Inhibitor (80,000 unit/ml)	5 µl	8.1 µl	11.3 µl	14.4 µl	17.5 µl	20.6 µl	1 unit/µl
Total	**360 µl**	**585 µl**	**811 µl**	**1,035 µl**	**1,260 µl**	**1,485 µl**	

Translation mixture

Thaw WEPRO®1240/WEPRO®7240 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.

Mix the reagents in a 2-ml microtube (*1).

Reagents	Number of samples						Final conc.
	1	2	3	4	5	6	
WEPRO®1240/ WEPRO®7240 (*2)	425 µl	675 µl	925 µl	1,175 µl	1,425 µl	1,675 µl	240 OD
Creatine kinase (20 µg/µl)	1.7 µl	2.7 µl	3.7 µl	4.7 µl	5.7 µl	6.7 µl	80 ng/µl
Total	**427 µl**	**678 µl**	**929 µl**	**1,180 µl**	**1,431 µl**	**1,682 µl**	

Note (*1): If bubbling occurs, ensure that the central area of the surface of the mixture in the 2-ml tube is free of bubbles.

Note (*2): To improve the purity of GST-tagged and His-tagged proteins, we recommend WEPRO®1240G and WEPRO®1240H, respectively.

Translation buffer

Thaw 1x SUB-AMIX®/1x SUB-AMIX® SGC on ice and mix gently by pipetting.

Place the reagent in a 50-ml tube

Reagents	Number of samples					
	1	2	3	4	5	6
1x SUB-AMIX®/ 1x SUB-AMIX® SGC	7 ml	12.5 ml	18 ml	23.5 ml	29 ml	34.5 ml

4-3. Reagents for purification

Prepare subscribed volumes of reagent mixtures according to the number of samples as shown in each table below. Dead volumes are included in the subscribed volumes.

GST-tagged protein purification using reduced glutathione buffer for elution

Required reagents

Reagents	Description
GST resin	Glutathione sepharose 4B of Cytiva. Replace the storage solution with wash buffer A to prepare 50% slurry.
Wash buffer A	Phosphate buffered saline
Elution buffer A	50 mM Tris-HCl, 10 mM reduced glutathione, pH 8.0

GST resin

Place the resin in a 14-ml tube.

Reagents	Number of samples					
	1	2	3	4	5	6
GST resin	1.4 ml	1.8 ml	2.2 ml	2.6 ml	3.0 ml	3.4 ml

Wash buffer A

Place the buffer in a 50-ml tube.

Reagents	Number of samples					
	1	2	3	4	5	6
Wash buffer A	7.5 ml	13.5 ml	19.5 ml	25.5 ml	31.5 ml	37.5 ml

Elution buffer A

Place the buffer in a 14-ml tube.

Reagents	Number of samples					
	1	2	3	4	5	6
Elution buffer A	1.4 ml	2.4 ml	3.4 ml	4.4 ml	5.4 ml	6.4 ml

GST-tagged protein purification using AcTEV™ protease for elution

Required reagents

Reagents	Description
GST resin	Glutathione sepharose 4B of Cytiva. Replace the storage solution with wash buffer A to prepare 50% slurry.
Wash buffer A	Phosphate buffered saline
Elution buffer B	1 unit/µl (final conc.) of AcTEV™ protease (Thermo Fisher Scientific) in phosphate buffered saline
Elution buffer D	Phosphate buffered saline

GST resin

Place the resin in a 14-ml tube.

Reagents	Number of samples					
	1	2	3	4	5	6
GST resin	1.4 ml	1.8 ml	2.2 ml	2.6 ml	3.0 ml	3.4 ml

Wash buffer A

Place the buffer in a 50-ml tube.

Reagents	Number of samples					
	1	2	3	4	5	6
Wash buffer A	7.5 ml	13.5 ml	19.5 ml	25.5 ml	31.5 ml	37.5 ml

Elution buffer B

Place the buffer in a 14-ml tube.

Reagents	Number of samples					
	1	2	3	4	5	6
Elution buffer B	0.9 ml	1.4 ml	1.9 ml	2.4 ml	2.9 ml	3.4 ml

Elution buffer D

Place the buffer in a 14-ml tube.

Reagents	Number of samples					
	1	2	3	4	5	6
Elution buffer D	0.9 ml	1.4 ml	1.9 ml	2.4 ml	2.9 ml	3.4 ml

GST-tagged protein purification using PreScission protease for elution

Required reagents

Reagents	Description
GST resin	Glutathione sepharose 4B of Cytiva. Replace the storage solution with wash buffer A to prepare 50% slurry.
Wash buffer A	Phosphate buffered saline
Elution buffer C	0.08 unit/µl (final conc.) of PreScission protease (Cytiva) in phosphate buffered saline
Elution buffer D	Phosphate buffered saline

GST resin

Place the resin in a 14-ml tube.

Reagents	Number of samples					
	1	2	3	4	5	6
GST resin	1.4 ml	1.8 ml	2.2 ml	2.6 ml	3.0 ml	3.4 ml

Wash buffer A

Place the buffer in a 50-ml tube.

Reagents	Number of samples					
	1	2	3	4	5	6
Wash buffer A	7.5 ml	13.5 ml	19.5 ml	25.5 ml	31.5 ml	37.5 ml

Elution buffer C

Place the buffer in a 14-ml tube.

Reagents	Number of samples					
	1	2	3	4	5	6
Elution buffer C	0.9 ml	1.4 ml	1.9 ml	2.4 ml	2.9 ml	3.4 ml

Elution buffer D

Place the buffer in a 14-ml tube.

Reagents	Number of samples					
	1	2	3	4	5	6
Elution buffer D	0.9 ml	1.4 ml	1.9 ml	2.4 ml	2.9 ml	3.4 ml

His-tagged protein purification using imidazole buffer for elution

Required reagents

Reagents	Description
His resin	Ni Sepharose 6 Fast Flow of Cytiva. Replace the storage solution with wash buffer B to prepare 50% slurry.
Solution A	600 mM imidazole pH 8.0
Wash buffer B	20 mM Na-phosphate pH 7.5, 0.3 M NaCl, 20 mM imidazole
Elution buffer E	20 mM Na-phosphate pH 7.5, 0.3 M NaCl, 500 mM imidazole

His resin

Place the resin in a 14-ml tube.

Reagents	Number of samples					
	1	2	3	4	5	6
His resin	1.2 ml	1.4 ml	1.6 ml	1.8 ml	2.0 ml	2.2 ml

Solution A

Place the solution in a 2-ml tube.

Reagents	Number of samples					
	1	2	3	4	5	6
Solution A	0.32 ml	0.52 ml	0.72 ml	0.92 ml	1.12 ml	1.32 ml

Wash buffer B

Place the buffer in a 50-ml tube.

Reagents	Number of samples					
	1	2	3	4	5	6
Wash buffer B	7.5 ml	13.5 ml	19.5 ml	25.5 ml	31.5 ml	37.5 ml

Elution buffer E

Place the buffer in a 14-ml tube.

Reagents	Number of samples					
	1	2	3	4	5	6
Elution buffer E	1.4 ml	2.4 ml	3.4 ml	4.4 ml	5.4 ml	6.4 ml

His-tagged protein purification using AcTEV™ protease for elution

Required reagents

Reagents	Description
His resin	Ni Sepharose 6 Fast Flow of Cytiva. Replace the storage solution with wash buffer B to prepare 50% slurry.
Solution A	600 mM imidazole pH 8.0
Wash buffer B	20 mM Na-phosphate pH 7.5, 0.3 M NaCl, 20 mM imidazole
Elution buffer F	1 unit/µl (final conc.) of AcTEV™ protease (Thermo Fisher Scientific) in elution buffer G
Elution buffer G	20 mM Na-phosphate pH 7.5, 0.3 M NaCl

His resin

Place the resin in a 14-ml tube.

Reagents	Number of samples					
	1	2	3	4	5	6
His resin	1.2 ml	1.4 ml	1.6 ml	1.8 ml	2.0 ml	2.2 ml

Solution A

Place the solution in a 2-ml tube.

Reagents	Number of samples					
	1	2	3	4	5	6
Solution A	0.32 ml	0.52 ml	0.72 ml	0.92 ml	1.12 ml	1.32 ml

Wash buffer B

Place the buffer in a 50-ml tube.

Reagents	Number of samples					
	1	2	3	4	5	6
Wash buffer B	7.5 ml	13.5 ml	19.5 ml	25.5 ml	31.5 ml	37.5 ml

Elution buffer F

Place the buffer in a 14-ml tube.

Reagents	Number of samples					
	1	2	3	4	5	6
Elution buffer F	0.9 ml	1.4 ml	1.9 ml	2.4 ml	2.9 ml	3.4 ml

Elution buffer G

Place the buffer in a 14-ml tube.

Reagents	Number of samples					
	1	2	3	4	5	6
Elution buffer G	0.9 ml	1.4 ml	1.9 ml	2.4 ml	2.9 ml	3.4 ml

5. Appendix

5-1. Protemist® DT II process descriptions for protein synthesis and purification

Protein synthesis

1.2-ml translation

Process	Description
Transcription	Add 45 µl of transcription mixture to plasmid DNA solution.
	Incubate at 37℃ for 6 hours.
Translation	Transfer 1.1 ml of 1x SUB-AMIX®/ 1x SUB-AMIX® SGC to each well of 24-well plate.
	Add 50 µl of WEPRO®1240/ WEPRO®7240 to transcripts.
	Transfer the whole mixture of WEPRO®1240/ WEPRO®7240 and transcripts to the bottom of each well of 24-well plate to form bi-layer mixes.
	Incubate at 15℃ for 20 hours.
	Keep the products at 4℃ after translation.

6-ml translation

Process	Description
Transcription	Add 225 µl of transcription mixture to plasmid DNA solution.
	Incubate at 37℃ for 6 hours.
Translation	Transfer 5.5 ml of 1x SUB-AMIX®/ 1x SUB-AMIX® SGC to each reaction cup.
	Add 250 µl of WEPRO®1240/ WEPRO®7240 to transcripts.
	Transfer the whole mixture of WEPRO®1240/ WEPRO®7240 and transcripts to the bottom of each reaction cup to form bi-layer mixes.
	Incubate at 15℃ for 20 hours.
	Keep the products at 4℃ after translation.

Protein purification after 6-ml translation

After translation, set aside 100 µl out of each 6-ml crude protein sample and store it at 4℃ for future reference. The remainder of the sample is subject to purification. Unless otherwise specified, 4℃ is the temperature of each step in the right column of the tables below.

Purification of GST-tagged protein using reduced glutathione buffer for elution

Process	Description
Binding	Add 400 µl of resin (50% slurry) to product solution in a reaction cup.
	Incubate for 60 min while shaking at 140 rpm.
	Remove unbound proteins (flow-through fraction) and store them at 4℃.
Wash	Add 2 ml of wash buffer A.
	Incubate for 5 min while shaking at 90 rpm.
	Waste wash fraction.
	Repeat this process 3 times.
Elution	Add 500 µl of elution buffer A.
	Incubate for 20 min while shaking at 70 rpm.
	Collect elution fraction and store it at 4℃.
	Repeat this process 2 times.

Purification of GST-tagged protein using AcTEV™ protease for elution

Process	Description
Binding	Same as above.
Wash	Same as above.
Elution	Add 500 µl of elution buffer B containing AcTEV™ protease.
	Incubate at 30℃ for 3 hours while shaking at 80 rpm.
	Collect elution fraction and store it at 4℃.
	Add 500 µl of elution buffer D to collect remaining target protein at the resin.
	Incubate at 30℃ for 5 min while shaking at 80 rpm.
	Collect elution fraction and store it at 4℃.

Purification of GST-tagged protein using PreScission protease for elution

Process	Description
Binding	Same as above.
Wash	Same as above.
Elution	Add 500 µl of elution buffer C containing PreScission protease.
	Incubate at 5℃ for 8 hours while shaking at 80 rpm.
	Collect elution fraction and store it at 4℃.
	Add 500 µl of elution buffer D to collect remaining target protein at the resin.
	Incubate at 5℃ for 5 min while shaking at 80 rpm.
	Collect elution fraction and store it at 4℃.

Purification of His-tagged protein using imidazole buffer for elution

Process	Description
Binding	Add 200 µl of solution A to product solution in a reaction cup.
	Incubate for 10 min while shaking at 140 rpm.
	Add 200 µl of resin (50% slurry) to product solution in a reaction cup.
	Incubate for 60 min while shaking at 140 rpm.
	Remove unbound proteins (flow-through fraction) and store them at 4℃.
Wash	Add 2 ml of wash buffer B.
	Incubate for 5 min while shaking at 90 rpm.
	Waste wash fraction.
	Repeat this process 3 times.
Elution	Add 500 µl of elution buffer E.
	Incubate for 20 min while shaking at 70 rpm.
	Collect elution fraction and store it at 4℃.
	Repeat this process 2 times.

Purification of His-tagged protein using AcTEV™ protease for elution

Process	Description
Binding	Same as above.
Wash	Same as above.
Elution	Add 500 µl of elution buffer F containing AcTEV™ protease.
	Incubate at 30℃ for 3 hours while shaking at 80 rpm.
	Collect elution fraction and store it at 4℃.
	Add 500 µl of elution buffer G to collect remaining target protein at the resin.
	Incubate at 5℃ for 5 min while shaking at 80 rpm.
	Collect elution fraction and store it at 4℃.

5-2. List of reagents available from CellFree Sciences

Reagents

Catalog No.	Product name	Specification	Size
CFS-TSC-5TB	5x Transcription Buffer	5x	1 ml
CFS-TSC-5TB-LM	5x Transcription Buffer LM	5x	1 ml
CFS-TSC-NTP	NTP Mix	25 mM	1 ml
CFS-TSC-ENZ	SP6 RNA Polymerase	80,000 unit/ml	1 set: 1 ml each
	RNase Inhibitor	80,000 unit/ml	
CFS-WGE-1240	WEPRO1240	240 OD	1 ml
CFS-WGE-1240G	WEPRO1240G *	240 OD	1 ml
CFS-WGE-1240H	WEPRO1240H **	240 OD	1 ml
CFS-WGE-7240	WEPRO7240	240 OD	1 ml
CFS-WGE-7240G	WEPRO7240G *	240 OD	1 ml
CFS-WGE-7240H	WEPRO7240H **	240 OD	1 ml
CFS-SUB	SUB-AMIX	4 parts, 40x each	1 set = 12.5 ml x 4 (500 ml as 1 x)
CFS-SUB- SGC	SUB-AMIX SGC	4 parts, 40x each	1 set = 12.5 ml x 4 (500 ml as 1 x)

*: To collect GST-tagged protein at a high purity. Yield of protein with WEPRO1240G/WEPRO7240G is slightly lower than that with WEPRO1240/WEPRO7240.

**: To collect His-tagged protein at a high purity. Yield of protein with WEPRO1240H/ WEPRO7240H is slightly lower than that with WEPRO1240/ WEPRO7240.

Reagent kits for Protemist® DT II

Catalog No.	Product name	Contents	Description
CFS-TRI-1240	WEPRO1240 Expression Kit	5x Transcription Buffer (400 µl)	One kit contains all reagents required for transcription and translation, enough for one run with 6 samples, 6 ml/sample.
		NTP Mix (25 mM, 200 µl)	
		SP6 RNA Polymerase (80,000 unit/ml, 30 µl)	
		RNase Inhibitor (80,000 unit/ml, 30 µl)	
		WEPRO1240 (240 OD, 1 ml x 2)	
		SUB-AMIX (40x, 1.1 ml x 4)	
		Creatine Kinase (20 µl, recommended to be used once)	
CFS-TRI-1240G	WEPRO1240G Expression Kit	WEPRO1240G (240 OD, 1 ml x 2)	Increased purity in GST-tagged protein purification
		Other reagents the same as in WEPRO1240 Expression Kit	
CFS-TRI-1240H	WEPRO1240H Expression Kit	WEPRO1240H (240 OD, 1 ml x 2)	Increased purity in His-tagged protein purification
		Other reagents the same as in WEPRO1240 Expression Kit	

Catalog No.	Product name	Contents	Description
CFS-TRI-7240	WEPRO7240 Expression Kit	5x Transcription Buffer LM (400 µl) NTP Mix (25 mM, 200 µl) SP6 RNA Polymerase (80,000 unit/ml, 30 µl) RNase Inhibitor (80,000 unit/ml, 30 µl) WEPRO7240 (240 OD, 1 ml x 2) SUB-AMIX SGC (40x, 1.1 ml x 4) Creatine Kinase (20 µl, recommended to be used once)	One kit contains all reagents required for transcription and translation, enough for one run with 6 samples, 6 ml/sample.
CFS-TRI-7240G	WEPRO7240G Expression Kit	WEPRO7240G (240 OD, 1 ml x 2) Other reagents the same as in WEPRO7240 Expression Kit	Increased purity in GST-tagged protein purification
CFS-TRI-7240H	WEPRO7240H Expression Kit	WEPRO7240H (240 OD, 1 ml x 2) Other reagents the same as in WEPRO7240 Expression Kit	Increased purity in His-tagged protein purification

5-3. Product-related information

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

5-4. Intellectual property rights

Our ENDEXT® technologies are covered by US Patent Nos. 6905843, 6869774 and 7919597, and other pending or equivalent patents.

5-5. Trademarks

Company names and product names in this manual are trademarks and registered trademarks of companies.
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5-6. Contact us

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