292-30021D
Nov. 2008

SHIMADZU BIOTECH

Transdirect *insect cell*

Instruction Manual

Read the instruction manual thoroughly before you use the product. Keep this instruction manual for future reference.

SHIMADZU CORPORATION
KYOTO JAPAN

ANALYTICAL & MEASURING INSTRUMENTS DIVISION

Introduction

Thank you for purchasing this product.
The following warranty information should be read carefully prior to use.

■ Warranty

- Term — The expiration date is printed on the outside of the reagent kit package. Be sure to use the product by this date.
- Scope — Please contact your Shimadzu representative for information about the scope of this product's warranty.
- Items Not Covered — The warranty does not cover problems resulting from:
  1) mishandling
  2) inappropriate storage
  3) external factors

NOTE Be aware that use of this product after the expiration date may not result in its original performance.

■ Purpose

- This product is designed for research. A special licensing contract must be signed when this product is used for other than research purposes.
- A special licensing contract must be signed when proteins synthesized by this product are offered commercially.

■ Notice

- This publication may not be reproduced in whole or in part without written permission from Shimadzu Corporation.
- Information in this document is subject to change without notice.
- The utmost care has been taken in preparing this document, however, if errors or omissions are detected, it may not be possible to correct them immediately.

■ Safety Conventions

In this instruction manual, the following safety-related signal words are used to explain the degree of hazard or possible damage.

⚠ **WARNING**	Indicates a potentially hazardous situation which, if not avoided, could result in serious injury or possibly death.
⚠ **CAUTION**	Indicates a potentially hazardous situation which, if not avoided, may result in minor to moderate injury* or equipment damage*.
NOTE	Emphasizes supplemental information that is provided to ensure the proper use of this product.

* Minor to moderate injury is an injury that does not require hospitalization or a long-term hospital stay.
* Equipment damage is significant damage to peripheral items other than the product, such as to the laboratory or to equipment.

■ Safety Guidelines

To safely use Transdirect *insect cell*, be sure to observe the following precautions.

⚠ **WARNING**	Generally, synthesized proteins and peptides may show unexpected toxicity or pathology. During experiments always wear or use safety equipment such as protective gloves, goggles, and masks, as well as safety-related items, including safety cabinets. When disposing of generated products, carry out autoclave and other deactivation procedures reliably.
⚠ **WARNING**	If reagents, reaction liquids or generated products contact the eyes or skin, or are swallowed or inhaled, consult a doctor immediately.
⚠ **CAUTION**	Be sure to follow all precautions established by your laboratory and perform all work in a safe manner.
⚠ **CAUTION**	Experiments using radio isotopes (RI) must be conducted in special RI experimental facilities. When handling RI, be sure to comply with all local, state/provincial or national laws and regulations.

- For storage and handling of reagents, be sure to refer to the relevant MSDS and observe all related laws and regulations.

Contents

Product Description

■ Overview

Transdirect *insect cell* is a cell-free protein synthesis system prepared from insect cells. It has the following features.

- Transdirect *insect cell* is a translation system for mRNA templates.
- Insect cell extract is prepared from *Spodoptera frugiperda* 21 (Sf21) insect cells, which are also widely used in the baculovirus expression system.
- The preparation method of insect cell extract and the concentration of the reaction components are optimized to increase protein synthesis efficiency (patent pending).
- Expression vector pTD1 (patent pending) includes the translational enhancer sequence (the 5' untranslated region derived from *Malacosma neustria* nucleopolyhedrovirus polyhedrin gene) suited to a cell-free protein synthesis system prepared from insect cells.

■ Kit Components

Transdirect *insect cell* (P/N 292-30000-95) consists of the components shown in Table 1, and an instruction manual (this document: P/N 292-30021).

Table 1: Kit Components and Storage (Equal to 40 reactions with 50 µL reaction volume)

Lid Color	Labels (Names)	Capacity	Number	Storage Conditions	Remarks
Yellow	Insect Cell Extract	210 µL	5	-80°C	
Blue	Reaction Buffer	630 µL	1	-80°C	Buffer solution, 19 amino acids (not including methionine), energy source etc.
Red	4 mM Methionine	50 µL	1	-20°C or below	Methionine (amino acid)
Green	0.5 µg/µL pTD1 Vector	10 µL	1	-20°C or below	Expression vector
White	0.5 µg/µL Control DNA	10 µL	1	-20°C or below	Control DNA is pTD1-β-Gal carrying β-galactosidase gene and linearized.

NOTE

- This product does not include transcriptional reactants. Prepare these separately.
- Store the Insect Cell Extract (yellow) and the Reaction Buffer (blue) at -80°C as soon as the product arrives. Be careful as the activity may be reduced or lost depending on storage conditions.
- Store the 4 mM Methionine (red), 0.5 µg/µL pTD1 Vector (green), and 0.5 µg/µL Control DNA (white) at -20°C or below (-80°C is also possible).
- Methionine is not included in the Reaction Buffer (blue) for effective RI labeling via ^{35}S Methionine. It is included separately.

Insect Cell Extract (Yellow)

(1) Do not mix Insect Cell Extracts with different Lot Nos. This may reduce the protein synthesis activity.
(2) Protein synthesis activity may differ between lots.
(3) Insect Cell Extract is sensitive to CO_2. After opening the package, avoid prolonged exposure to CO_2.

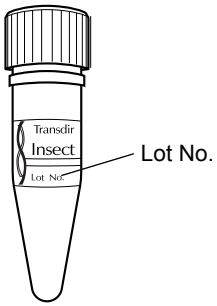


Figure 1: Lot No.

◆ Freezing and Thawing

Do not freeze and thaw the Insect Cell Extract (yellow) more than 8 times.
Be sure to thaw the Insect Cell Extract on ice. Use the Insect Cell Extract soon after thawing, and immediately after use store it again at -80°C.

■ User-Supplied Items

Tools and Equipment

- Thermostatic chamber (adjustable to 25°C and 37°C)
- Spectrophotometer
- Pipeter
- Sterilized tips and tubes
- Disposable gloves
- Disposable gel filtration column (if necessary)

Reagents

- Large-Scale mRNA Synthesis Kit (T7)
- Restriction endonuclease used for linearization (Ex. *Hind* III, *Not* I and others)
- Sterilized distilled water
- TE
- 3M Potassium Acetate (pH5.5)
- TE saturated Phenol

- Chloroform

- Ethanol

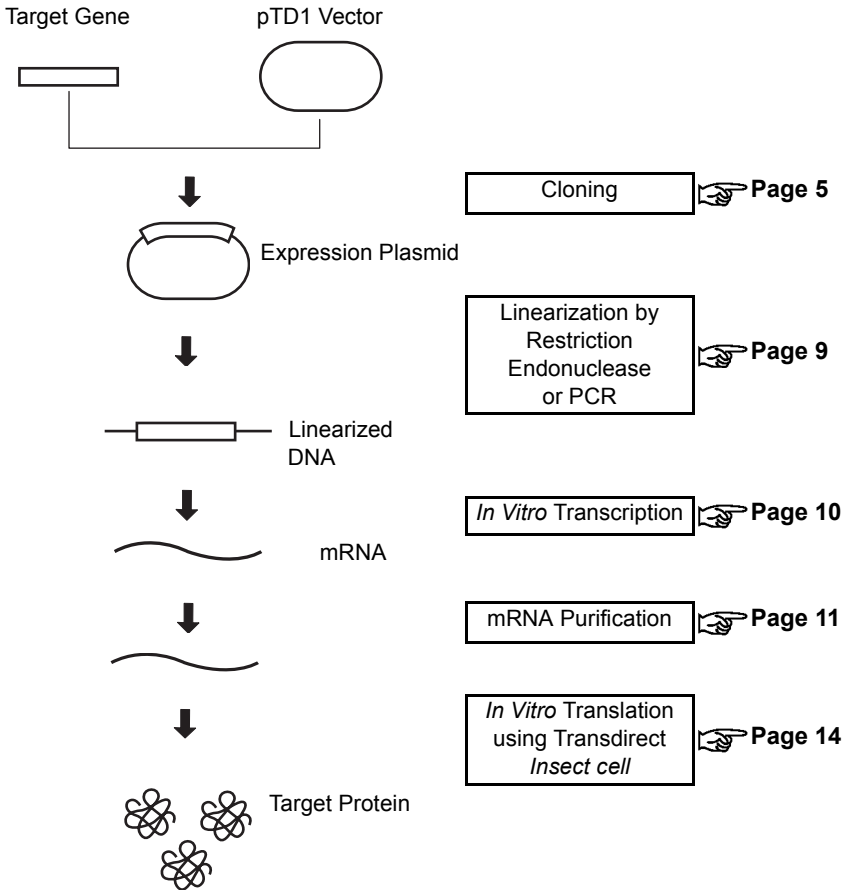
- 70% Ethanol

■ Using Transdirect *insect cell*

When using Transdirect *insect cell*, be aware of the following points.

(1) Synthesis may not be possible depending on the type of protein.
(2) When using only Transdirect *insect cell*, N-glycosylation and other post-translational modifications via endoplasmic reticular membranes do not occur.

■ Workflow



The following bulk kits are available for large-scale preparation.

P/N	Product
292-30000-92	Bulk kit20 (equal to 20kits)
292-30000-93	Bulk kit50 (equal to 50kits)
292-30000-94	Bulk kit100 (equal to 100kits)

Protocols

■ Preparation of DNA Template for *In Vitro* Translation

pTD1 Vector

The pTD1 Vector is a vector optimized for cell-free protein synthesis using Transdirect *insect cell* (Figure 2). The pTD1 Vector includes all factors involved in mRNA synthesis and protein synthesis, including the T7 promoter sequence required for mRNA synthesis, the polyhedrin 5' untranslated region (polyhedrin 5' UTR) which enhances translation, and multiple cloning sites (MCS).
An ampicillin-resistant gene is also included. When cultivating transformants, add these to the culture medium until the ampicillin concentration reaches 50 µg/mL to 100 µg/mL.

The pTD1 Vector's complete sequence is registered in the following DNA databank.
DDBJ/GenBank®/EMBL Accession Number: AB194742

NOTE It is also possible to carry out protein synthesis from other plasmids. However, the amount of synthesized protein may be reduced. Cloning of target genes to the pTD1 Vector is recommended. In addition, since polyhedrin 5' UTR for translational enhancer has been inserted in the pTD1 Vector, the introduction of a cap structure utilizing Cap Analog during mRNA synthesis is not required.

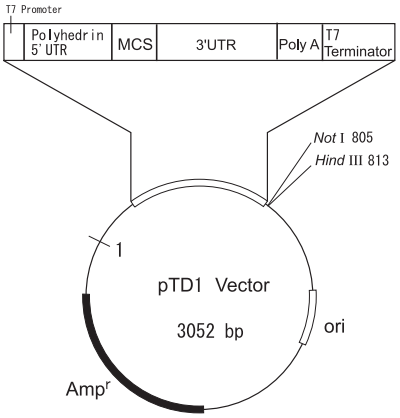


Figure 2: pTD1 Vector Map

Table 2: pTD1 Vector Sequence Reference Points

T7 RNA polymerase promoter	240-258
Polyhedrin 5' untranslated region	259-304
Multiple cloning region	305-343
3' Untranslated region	359-731
PolyA region	736-757
T7 transcription terminator	758-805
ColE1 ori	835-1104
β-lactamase (Amp^r) coding region	1992-2852

GCAGATTGTA CTGAGAGTGC ACCATATGCG GTGTGAAATA CCGCACAGAT GCGTAAGGAG 220
Forward primer

AAAATACCGC ATCAGGCCTT AATACGACTC ACTATAGGAG TATTTTTATT CTTTCGTAAA 280
T7 Promoter **Polyhedrin 5'UTR**

***Sma* I**
AAAATTAGAA AAATAAAATA TAAAGATATC GAATTCGAGC TCGGTACCCG GGGATCCTCT 340
***Eco* RV** ***Eco* RI** ***Sac* I** ***Kpn* I** ***Bam* HI** ***Xba* I**

AGAGTCGGGC GGCTGTAAAA CACGATACAT TGTTATTAGT ACATTTATTA AGCGCTAGAT 400

TCTGTGCGTT GTTGATTTAC 420
Reverse primer

Figure 3: pTD1 Vector Multiple Cloning Site

Cloning Site of Target Genes

When cloning targeted genes to the pTD1 Vector, insert the initiating codon of the target genes downstream of the polyhedrin 5' UTR. The productivity will be reduced if there is a long distance between the initiating codon and the polyhedrin 5' UTR. Inserting into the *Eco* RV site is recommended whenever possible.

Expression Plasmid Construction

The following shows the general method of constructing expression plasmids when target genes are inserted into the *Eco* RV site.

1 The target gene's ORF[*1] is amplified by PCR[*2].
At this point, the N-terminal primer should have the sequence from the initiating codon. As for C-terminal primers, with the exception of blunt end cloning, introduce the restriction endonuclease site's sequence at the 5'-end. Depending on the type of restriction endonuclease, digestion may not be possible if there is no additional sequence at the 5'-end. To carry out efficient digestion by restriction endonuclease, addition of 2 bases at the 5'-end of the C terminal primer is recommended. Insert a stop codon following the additional sequence and restriction endonuclease site.

NOTE *1 With PCR from eukaryotic genomes, carry out PCR from cDNA, since intron may be included.
*2 Select a high-fidelity enzyme in order to avoid errors from PCR. In addition, for blunt-end cloning of the initiating codon, Shimadzu recommends use of PCR enzymes with low Terminal Transferase activity (e.g. Toyobo Ltd. KODplus [Code No. KOD-201]).

NOTE The description in this document does not indicate patent licensing with respect to PCR. Follow the terms of your PCR reagents.

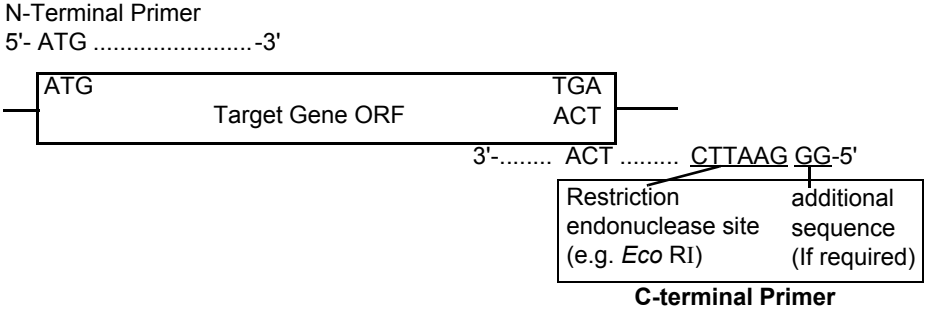


Figure 4: Primer Construction Methods

2 Phosphorylate the PCR product.

3 Digest using the same restriction endonuclease as the restriction endonuclease site introduced in the PCR products.

4 Digest using a restriction endonuclease having the same cohesive terminal as the restriction endonuclease where pTD1 was introduced to the *Eco* RV insertion site and the target gene.

5 Using the above, insert the target gene into the pTD1 Vector.

◆ Insert Check

NOTE Shimadzu recommends using the following sequence primer. (Annealing temperature: 50°C)

- Forward primer (N-terminal):
  5'-GCAGATTGTACTGAGAGTG-3' (161-179)
- Reverse primer (C-terminal):
  5'-ACAACGCACAGAATCTAGC-3' (412-394)

■ mRNA Preparation

Linearization of DNA Template

NOTE

- Linearize the DNA template by digestion with an appropriate restriction endonuclease at the downstream of the terminator sequence (Ex. *Hind* III, *Not* I). After linearizing with the restriction endonuclease, extract with phenol/chloroform and precipitate with ethanol to prevent RNase contamination.
- PCR-generate DNA template can also be used in transcription reactions. In this case also, extract with phenol/chloroform and precipitate with ethanol. Use of the following sequence primer is recommended.
  (Annealing temperature: 50°C)

1) Forward primer (N-terminal):
   5'-GCAGATTGTACTGAGAGTG-3' (161-179)
2) M13 series reverse primer (C-terminal):
   5'-GGAAACAGCTATGACCATG-3' (845-827)

Table 3: Restriction Endonuclease Appropriate for pTD1Vector Linearization

Restriction Endonuclease	Breakage Position
Cfr 10I	2145
Eco 52I	806
Eco T14I	769
Hind III	813

Restriction Endonuclease	Breakage Position
Nde I	183
Not I	805
Pvu II	994
Sca I	2543
Stu I	234

In Vitro Transcriptional Reaction

Items Supplied by User

- Thermostatic chamber (capable of incubation at 37°C)

NOTE

- It has been confirmed that protein synthesis is possible using mRNA prepared using the following kits. Synthesize mRNA according to the steps given in each instruction manual.
- Be sure to wear gloves for all procedures.

Table 4: Large-Scale mRNA Synthesis Kit

Name of Product	Manufacturer	Code No.
AmpliScribe™ T7-*Flash* ™ Transcription Kit	Epicentre	ASF3257
AmpliScribe™ T7 Transcription Kit	Epicentre	AS3107
CUGA® 7 *in vitro* Transcription Kit	Nippon Genetech Co., Ltd.	304-14641
MEGA script® T7 High Yield Transcription Kit	Ambion	1334
RiboMAX™ Large Scale RNA Production System-T7	Promega	P1300
RNAMaxx™ High Yield Transcription Kit	Stratagene	200339
ScriptMAX™ Thermo T7 Transcription Kit	Toyobo Ltd.	TSK101
T7 RiboMAX™ Express Large Scale RNA Production System	Promega	P1320

(Product names are arranged in alphabetical order.)

mRNA Purification

This section explains the following two mRNA purification methods.
Method A, in which salt and unincorporated NTPs are removed by gel filtration, is recommended for synthesis requiring high repeatability and stable reactions.
Method B provides a convenient method of purification. Since this method does not completely remove unincorporated NTPs, the mRNA concentration will appear to rise. To obtain the maximum efficiency, Shimadzu recommends you perform testing to determine the optimal concentration of mRNA.

A	Purification Method Using Gel Filtration Column and Ethanol Precipitation (☞ Page 11)
B	Purification Method by Ethanol Precipitation (☞ Page 13)

◆ Purification Using Gel Filtration Column and Ethanol Precipitation

Materials to be Supplied by User

- Prepackaged disposable gel filtration column*1
- 3M potassium acetate (pH 5.5)*2
- Ethanol
- 70% Ethanol
- Sterilized distilled water (distilled water, treated in an autoclave for 20 minutes at 120°C)

NOTE *1 GE Healthcare' NICK™ Columns (Code No. 17-0855-01) are recommended.

*2 Use potassium acetate for the salt in the ethanol precipitation. If any other salt is used, the protein synthesis efficiency may be reduced.

Procedures

The following explains the mRNA purification method described in the NICK™ Columns protocol. For details, refer to the GE Healthcare instruction manual.

NOTE Be sure to wear gloves for all procedures.

1 Remove the column's cap and discard the solution.

2 Rinse the column with 3 mL of sterilized distilled water, and discard the sterilized distilled water.

3 Remove the column tip cap and place it in the stand.

4 For gel equilibrium, add 3 mL of sterilized distilled water, and flush completely.

5 Apply the mRNA solution on top of the gel, up to a maximum of 100 µL, and then flush completely.

6 Add 400 µL of sterilized distilled water, and then flush completely.

7 To receive the mRNA solution, place a 1.5 mL tube below the column.

8 Add 400 µL of sterilized distilled water and collect the mRNA solution. (Approx. 400 µL can be reclaimed.)

9 Add 40 µL of potassium acetate and 950 µL of ethanol to the collected mRNA solution. Mix thoroughly and centrifuge for 20 minutes at 15,000 rpm, 4°C.

10 Discard the supernatant and then rinse with 70% ethanol. Depending on the mRNA synthesis scale, dissolve in 20 µL to 100 µL of sterilized distilled water.

NOTE Adjust the mRNA concentration to 2 µg/µL.

◆ **Purification Method by Ethanol Precipitation**

Procedures

1 Adjust with sterilized distilled water so as to obtain a 400 µL mRNA synthesis solution.

2 Purify the solution according to procedures 9 and 10 in the "Purification Using Gel Filtration Column and Ethanol Precipitation". (☞ Page 11)

NOTE In this case, as dissolution is difficult due to unincorporated NTPs, dissolve with sterilized distilled water, without complete drying after rinsing.

Measuring mRNA Concentration

After purification, measure the light absorbance of the mRNA solution and determine the concentration. Dilute the mRNA solution with TE (100 to 400 times), and then measure the light absorbance using a quartz cell. Use TE as the blank.

mRNA concentration (µg/µL) = A_{260} value × 0.04 × dilution rate

■ *In Vitro* Translational Reaction

In Transdirect *insect cell*, the reaction components have been optimized to obtain a high protein synthesis efficiency. It is also compatible with RI labeling and fluorescent labeling experiments.

Materials to be Supplied by User

- Sterilized distilled water (distilled water, treated in an autoclave for 20 minutes at 121°C)
- Thermostatic chamber (capable of incubation at 25°C)
- Purified mRNA

When Conducting RI Labeling Experiments:

- [^{35}S] Methionine

When Conducting Fluorescent Labeling Experiments

- Promega FluoroTect™ Green$_{Lys}$ in vitro Translation Labeling System (Code No. L5001)

Standard Protocols

The following is the reaction components at a scale of 50 µL. Scaling up or down is possible in relation to the application.

Table 5: 50 µL Scale Reaction Components

mRNA	16 µg
Reaction Buffer (blue)	15 µL
Insect Cell Extract (yellow)	25 µL
4 mM Methionine (red)	1 µL
Sterilized distilled water	Adjust to 50 µL

◆ Procedures

NOTE Wear gloves for all procedures, and be sure to perform them on ice, or at 4°C.

1 Remove the required number of Insect Cell Extract vials (yellow) from -80°C storage, and thaw on ice.

NOTE
- Thaw for approximately 30 minutes. Use the extract soon after thawing, and immediately after use re-store at -80°C.
- Reaction Buffer (blue) and 4 mM Methionine (red) can be thawed at room temperature. After thawing, place the vial on ice. After use, immediately store it again at the prescribed temperature. (☞ Page 1)

2 Mix the substances in a newly prepared tube.

3 Add mRNA, lightly tap and then spin down. Synthesis reaction will start.

4 Incubate the reaction tube in a thermostatic chamber set to 25°C for 5 hours.

5 After the synthesis reaction is complete, immediately place the reaction tube on ice, and use it for the intended research.

Standard Protocols for Protein Labeling

Labeling methods are shown using [^{35}S] Methionine and Promega FluoroTect™ Green$_{Lys}$ in vitro Translation Labeling System (Code No. L5001).
The following is the reaction components at a scale of 50 µL. Scaling up or down is possible in relation to the application.

NOTE It is possible to use [^{3}H] Leucine for the RI labeling experiments.

If such is the case, add 4 mM Methionine to have reaction as you do in the standard protocols.

For the detection, keep in mind that exposure time should be longer than usual.

Table 6: 50 µL Scale Reaction Components (Standard Protocols for Protein Labeling)

Labeling Method	[^{35}S] Met Label	FluoroTect™Label
mRNA	16 µg	16 µg
Reaction Buffer (blue)	15 µL	15 µL
Insect Cell Extract (yellow)	25 µL	25 µL
[^{35}S] Methionine (1200 Ci/mmoL) 10 mCi/mL	1 µL	——
4 mM Methionine (red)	——	1 µL
FluoroTect™ Green$_{Lys}$tRNA	——	1 µL
Sterilized distilled water	Adjust to 50 µL	

CAUTION RI labeling experiments must be conducted in special RI experimental facilities. When handling RI, be sure to comply with all local, state/ provincial or national laws and regulations.

◆ Procedures

Carry out the synthesis reaction in accordance with standard protocols (☞ Page 15) procedures 1 to 5.

Using RI-Labeling to Detect Synthesized Proteins

After resolving a portion of the reaction mixture by SDS-PAGE, RI-labeled protein can be detected as bands using autoradiography.

1. Perform CBB staining of gel after SDS-PAGE.
2. After destaining with CBB destaining solution, dry the gel.
3. Perform autoradiography using the dried gel.

NOTE
- X-ray film exposure should be from 6 hours to 1 day.
- To improve sensitivity, use a radiosensitizing agent (e.g. Amersham Biosciences Amplify Fluorographic Reagents (Code No. NAMP100)) and perform a fluoroscopy exposure at -80°C.

Using Fluorescent Labeling to Detect Synthesized Proteins

After resolving a portion of the reaction mixture by SDS-PAGE, protein that has been fluorescent labeled using Promega FluoroTect™ $Green_{Lys}$ in vitro Translation Labeling System (Code No. L5001) can be detected as bands by a fluorescent image analyzer.

Positive Control Translational Reaction Using β-Galactosidase

The 0.5 µg/µL Control DNA (white) carrying β-galactosidase provided is template DNA for positive control reactions. This DNA has already been linearized.

1. Using 0.5 µg/µL Control DNA (white), prepare the β-galactosidase mRNA as explained in "mRNA Preparation".(☞ Page 9)
2. Using β-galactosidase mRNA, synthesize β-galactosidase as explained in "Standard Protocols".(☞ Page 14)

Detection of Positive Control β-Galactosidase

The following are quantitative and qualitative methods for confirming the synthesis of β-galactosidase as positive control.

◆ Quantitative Method

Materials to be Supplied by User

- Promega β-Galactosidase Enzyme Assay System with Reporter Lysis Buffer (Code No. E2000)
- Spectrophotometer (Ex. Shimadzu Corporation Biospec-mini)

Procedures

The following explains the quantitative method for confirming the synthesis of β-galactosidase. Refer to the Promega instruction manual for details on creation of the calibration curve and the calculation of activity values.

1. Add 490 µL of 1 × Reporter Lysis Buffer to 10 µL of synthesis reaction liquid, until the solution is diluted 50 times.
2. Take 10 µL of this diluted solution and add 140 µL of 1 × Reporter Lysis Buffer and 150 µL of Assay 2 × Buffer. Then, incubate the solution for 30 minutes at 37°C.
3. After 30 minutes, quickly add 500 µL of 1M Sodium Carbonate, thereby stopping the reaction.
4. Using a spectrophotometer, measure the light absorbance at 420 nm. Multiply this value by the dilution factor of 50, and calculate the β-galactosidase activity U/mL using the numerical value obtained from the calibration curve.

NOTE 10 U/mL of β-galactosidase can be synthesized by carrying out the positive control reaction.

◆ Qualitative Method

Materials to be Supplied by User

- Reaction mixture after protein synthesis in accordance with protocol
- X-Gal solution (20 mg/mL dimethyl formamide 

/ 

)

Reaction Mixture	3 µL
X-Gal Solution	1 µL
Sterilized Distilled Water	16 µL
Total	20 µL

Procedure

1 Incubate at 37°C for 20 minutes.

2 If protein synthesis is completed normally, reaction solution will turn blue.

- If no mRNA is added, the reaction solution will remain colorless.

Appendix

■ Troubleshooting

Problem	Cause	Solution
Low or no recovery of plasmid	Strain is inappropriate.	DH5α™ (Invitrogen Code No. 18258-012) is recommended as the *E. coli* for plasmid preparation. Additionally, culturing with LB culture media is recommended.
Low transcriptional efficiency	Salt or some inhibitors are mixed with the template DNA.	Perform phenol/chloroform extraction and ethanol precipitation, and then wash carefully with 70% ethanol. (☞ Page 9)
	mRNA has become degraded.	Completely deactivate the RNase in the template DNA solution, using procedures such as phenol/ chloroform extraction.
Low translational efficiency of control protein	Kit storage conditions are inappropriate.	Store the Insect Cell Extract (yellow) and Reaction Buffer (blue) at -80°C. Store the 4 mM Methionine (red), 0.5 µg/µL pTD1 Vector (green) and 0.5 µg/µL Control DNA (white) at -20°C or below. (☞ Page 1)
	mRNA has become degraded.	Completely deactivate the RNase in the mRNA solution, using procedures such as phenol/ chloroform extraction.
		Add 50 units RNase inhibitor (from human placenta) for a 50 µL reaction volume. This may improve translational efficiency. (Recommended product: Takara Bio Inc. Code No. 2310A, Promega Code No. N2611)
	Magnesium or other salts are mixed with the mRNA solution.	Salt concentration in the reaction mixture has been optimized. The protein productivity may be reduced due to salt mixed from the mRNA solution. Use ethanol precipitation to remove the salt.

Problem	Cause	Solution
Low translational efficiency of control protein	Ethanol is mixed in the mRNA solution.	Protein productivity may be reduced due to ethanol contamination. Avoid ethanol contamination as much as possible during mRNA preparation.
	mRNA concentration is incorrect.	Since unincorporated NTPs are not completely removed using ethanol precipitation, mRNA concentration appears to rise. Test to identify what is the optimal mRNA additive quantity. (☞ Page 11)
	Freezing/thawing has been performed many times.	Perform no more than 8 freezing and thawing cycles. In addition, be sure to thaw the product on ice. Use the product soon after thawing. After use, store it immediately at -80°C. (☞ Page 2)
A sufficient quantity of control protein has been synthesized. However, the target protein has not been synthesized, or the amount synthesized is too small.	Cloning site is incorrect.	When inserting the target genes into the vector, insert the cDNA ORF initiating codon near the enhancer sequence as much as possible. Insertion at the *Eco* RV site is recommended. (☞ Page 9 Table 3)
	Vector is incorrect.	Use the included vector, 0.5 µg/µL pTD1 Vector (green).
	Restriction endonuclease used for linearization is incorrect.	Use the restriction endonuclease described in Page 9 Table 3, which does not digest the insert.
	Mutations exist in the insert.	Confirm the insert sequence.
	Initiating codon has been lost.	Confirm the insert sequence.

Problem	Cause	Solution
Activity is not detected.	Protein has not folded correctly.	Correct folding may not occur if special chaperones are involved in folding. In addition, aggregation may result with membrane proteins and other highly hydrophobic proteins.
	Disulfide bonds have not formed.	Since the protein synthesis reaction is carried out under reduction conditions, activity reduction or loss may be evident in the case of proteins for which sulfide bonds are required for activity. Consider to purchase Transdirect non-reducing version (spcial order produce).
Protein cannot be purified.	Effect from salt.	Desalting is necessary when purifying with an ion exchange column, since the protein synthesis reagent contains high concentrations of salt.
Background levels are high during RI detection.	Exposure time is too long.	If the exposure time is too long, non-specific bands that are not targeted may be detected. Adjust the exposure time.
	RI additive amount is incorrect.	If the RI additive amount is too large, non-specific bands that are not targeted may be detected. If the additive amount is too small, exposure will require a long time. Adjust the additive amount.

■ References

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J. Biosci. Bioeng., 2006, 102, 69-71

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Proteomics, 2006, 6, 4486-4495

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Preparation of N-acylated proteins modified with fatty acids having a specific chain length using an insect cell-free protein synthesis system
Biosci. Biotechnol. Biochem., 2007, 71, 261-264

Suzuki, T. *et al.*
Protein prenylation in an insect cell-free protein synthesis system and identification of products by mass spectrometry
Proteomics, 2007, 7, 1942-1950

Ezure, T. *et al.*
Expression of proteins containing disulfide bonds in an insect cell-free system and confirmation of their arrangements by MALDI-TOF mass spectrometry
Proteomics, 2007, 7, 4424-4434

■ Ordering MSDS

MSDS (Material Safety Data Sheet) for each chemical substance is provided free upon request. Contact the following Technical Support address, or visit the Shimadzu website where copies are posted.
(URL: http//:www.shimadzu-biotech.jp/reagents/trans/d_seet_e.pdf)

■ Technical Support

The Clinical & Biotechnology Business Unit Life Science Business Department, Analytical & Measuring Instruments Division, Shimadzu Corporation
(E-mail: t-direct@shimadzu-biotech.jp)