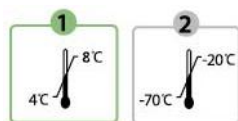


User's Guide

ExiProgen[™] EC Protein Synthesis Kit

REF

K-7300~7302



ExiProgen[™] EC- Protein Synthesis Kit

User's Guide

Version No.: 2.1 (2016-11)

Please read all the information in booklet before using the kit



Bioneer Corporation
8-11, Munpyeongseo-ro
Daedeuk-gu, Daejeon 34302
Korea

Tel: +82-42-930-8777

Fax: +82-42-930-8688

Email: sales@bioneer.com

www.bioneer.com

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Safety warning and Precaution

ExiProgen™ EC Protein Synthesis Kit is developed and supplied for research purposes only. Certain applications possible with this kit may require special approval by appropriate local and/or national regulatory authorities in the country of use.

Wear appropriate protection when handling any irritant or harmful reagents. The use of a laboratory coat, protective gloves and safety goggles are highly recommended. For more information please consult the appropriate Material Safety Data Sheet (MSDS).








Warranty and Liability

All BIONEER products undergo extensive Quality Control testing and validation. BIONEER guarantees quality during the warranty period as specified, when following the appropriate protocol as supplied with the product. It is the responsibility of the purchaser to determine the suitability of the product for its particular use. Liability is conditional upon the customer providing full details of the problem to BIONEER within 30 days.

Patent

ExiProgen™ and its kits are protected by the patents KR10-2011-0085824, PCT/KR2012/006715.

Explanation of Symbols

 REF	Catalog Number	 Contains sufficient for (n) tests	 USE BY
 LOT	Batch code	 Caution, Consult accompanying documents	 Temperature Limitation
 Manufacturer		 Caution, Potential Biohazard	 Do NOT REUSE
 Consult Instruction For Use		 Research Use Only	

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1. Introduction

Proteins are one of the essential components in the vital reactions *in vivo* and *in vitro*, with various functions as enzymes, hormones, and structural proteins. The research on the roles and structures of proteins has been done actively in the post-genomic era, starting with the production of proteins of interest by using recombinant DNA technology.

In order to produce recombinant proteins people generally transform various host cells, such as *E. coli*, yeast, and mammalian cells with a vector that contains a piece of DNA necessary for the expression of a target protein. The transformed cells that demonstrate a stable expression of the recombinant protein are selected by the selection marker co-inserted into the host cells with a target DNA. The recombinant proteins are produced during the culture process of the selected transformed cells and after the culture collected and purified from either the cell lysate or the culture solution. The whole process of cell-based protein synthesis comprising from recombination and transformation up to purification is usually a tedious and labor intensive job. Additionally for the toxic proteins to the host cells it is very hard to express those proteins in a cell-based system. Therefore those toxic proteins are obtained through repeated trials and errors under various conditions, which takes several days or even several months.

To overcome the limitations of cell-based system, a cell-free protein expression method and its related products have been developed to accomplish protein synthesis inside a reaction vessel in a short period of time. This method only requires to add cell lysate and template DNA, either of an expression vector or a PCR product, to protein expression solution that contains amino acids, nucleic acids and energy source in the reaction vessel. Then the target recombinant protein is expressed during a reaction at appropriate temperature. This method has the advantage of quite shorter processing time, over the cell based method, as well as of expression of toxic proteins to the cells.

The cell-free protein expression method has been used in functional research on new genes and comparison studies on functional activities of various mutant proteins. It also has broad applications such as research on the mechanism of protein-protein interaction and on the active site of proteins.

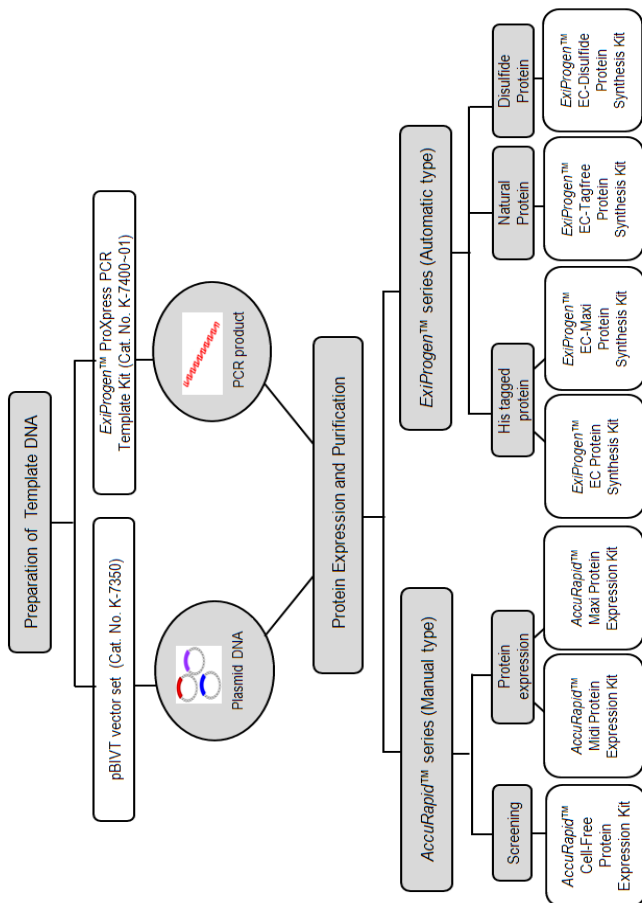
2. The *ExiProgen*™ protein synthesis system

Bioneer has developed the *ExiProgen*™ that combines cell-free protein expression system and highly efficient purification system. The *ExiProgen*™ expresses and purifies various proteins within a day in a fully automated fashion and provides the target protein with high purity and amount of from micrograms up to milligrams. Furthermore, the *ExiProgen*™ can extract DNA and RNA from various biological samples within an hour.

The *ExiProgen*™ expressed a target protein from a template DNA and reagents provided by Bioneer and purifies the expressed protein using affinity reactions between the Ni-NTA magnetic nanoparticles and the 6x histidine tag attached at the end of the protein. The entire process of expression and purification is automated. The only requirement is to construct a user's own template DNA and to install a protein synthesis kit in the *ExiProgen*™.

Bioneer currently provides various protein synthesis kits that have been optimized for many different proteins. Therefore, researchers can simply execute a protocol already programmed in the *ExiProgen*™ along with installing the kit of interest.

Bioneer's cell-free protein synthesis products are divided into three groups (a figure on page 4): 1) products for the generation of template DNA; 2) *AccuRapid*™ products for manual expression of proteins; 3) *ExiProgen*™ products for automated protein expression and purification using *ExiProgen*™ instrument.



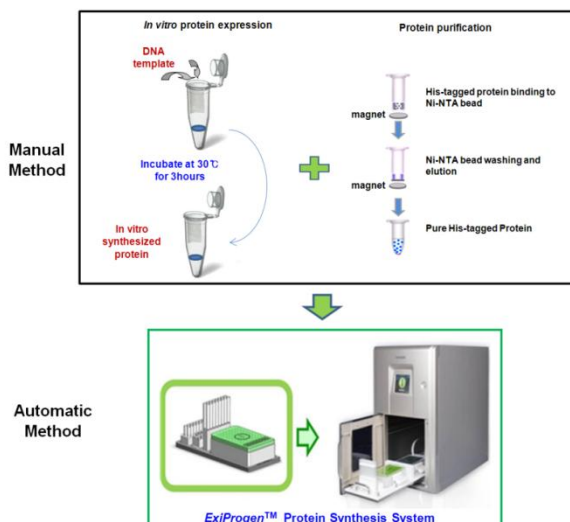
3. ExiProgen™ EC Protein Synthesis Kit

3.1. Overview

This product consists of a cell-free protein expression and Histidine-tag based affinity purification system. This product is intended to be used with Bioneer's automated ExiProgen™ system for obtaining up to 16 different types of highly pure proteins within 6 hours of adding DNA. The principle of the product is explained in Figure 1.

This product has applied automation to *in vitro* protein expression and magnetic particle-based purification methods. Unlike manual methods which can yield disparate results depending on user, this system provides robust reproducibility due to the automation.

Figure 1. Principle of ExiProgen™ Protein Synthesis System

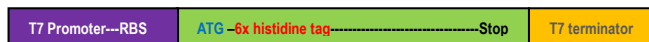


3.2. Prepare of template DNA

A protein expression vector can be used as template DNA for the ExiProgen™ EC Protein Synthesis Kit. Figure 2 shows the typical structure of template DNA for the ExiProgen™. The template DNA should have the structure of “T7 promoter – Ribosome binding site (RBS) – Target gene – T7 terminator”. The target gene should also have start codon and stop codon for protein translation, as well as 6x histidine tag at either 5’– or 3’–terminal for the ensuing purification.

Figure 2. Structure of template DNA

1) NH constructs (5’ end 6x histidine tagged template)



2) CH constructs (3’end 6x histidine tagged template)



For cloning of a target gene, one can use pBIVT vector from Bioneer, which is a protein expression vector for *in vitro* translation (Cat. No. K-7350). A detailed explanation on pBIVT vector is provided in Appendix 1 of the manual. pK7, pIVEX, and pET-series vectors could be used for this purpose.

In order to obtain the maximum amount of synthesized protein, it is recommended that the DNA sequence of target gene be optimized to the codon usage for *E. coli*. Bioneer provides Gene Synthesis Service to a customer if the target gene is not optimized to *E. coli*. Throughout the service a customer can get codon-optimized target gene cloned into pBIVT vector (or other *in vitro* translation vector a customer selects). Please refer to Bioneer’s homepage (www.bioneer.com) for more information.

3.3. Kit contents and storage

ExiProgen™ EC Protein Synthesis Kit ①			
Catalog number	K-7300 (16 reactions)	K-7301 (32 reactions)	K-7302 (96 reactions)
Cartridge ①	96 well x 1 ea	96 well x 2ea	96 well x 6ea
Disposable filter tip	2 packs (8 ea/pack)	1 pack (33 ea/pack)	3 packs (33 ea/pack)
Protection cover	1 ea	2 ea	6 ea
ExiProgen™ EC Protein Synthesis Kit ②			
Catalog number	K-7300 (16 reactions)	K-7301 (32 reactions)	K-7302 (96 reactions)
Cartridge ②	96 well x 1 ea	96 well x 2ea	96 well x 6ea
<i>E. coli</i> extract	8-tube strip x 2 ea	8-tube strip x 4 ea	8-tube strip x 12 ea
Positive control DNA	1.5mL tube x 1 ea	1.5mL tube x 1 ea	1.5mL tube x 1 ea
Elution tube/cap	8-tube strip x 2 ea	8-tube strip x 4 ea	8-tube strip x 12 ea

ExiProgen™ EC Protein Synthesis Kit consists of Kit ① and Kit ②.

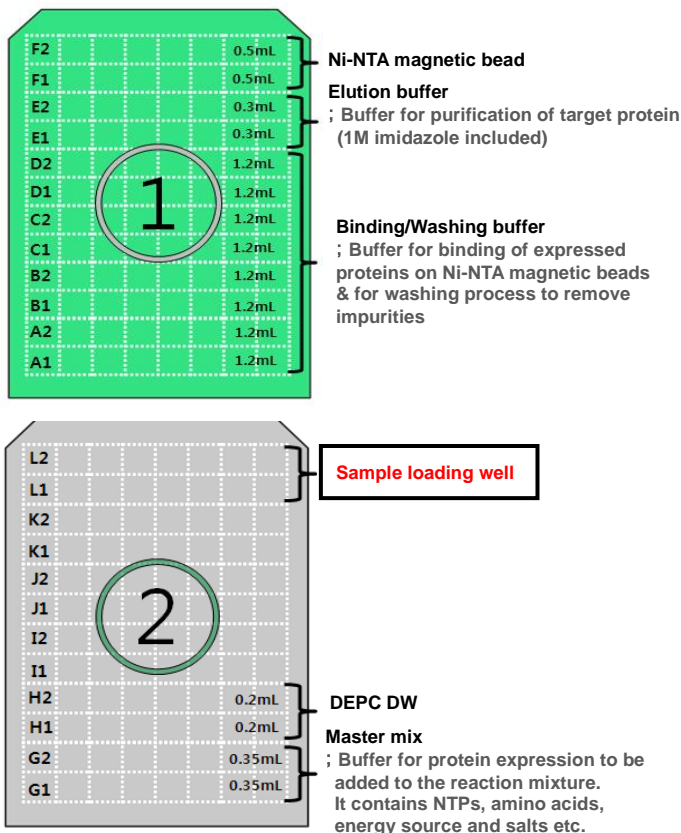
The Kit ① includes Cartridge ①, Disposable filter tip. Cartridge ① contains buffers to be used for protein purification and should be stored in a refrigerator (4–8°C).

The Kit ② includes Cartridge ②, *E. coli* extract, Elution tube/cap and a tube of Positive Control DNA. Cartridge ② contains Master mix and DEPC DW for protein expression. The Kit ② should be stored at –20°C or below, and *E. coli* extract is strongly recommended to locate at a –70°C deep freezer.

The Cartridges of this kit are covered with 3-ply sealing films in order to prevent cross-contamination, evaporation, or leakage of solutions inside. All of the plastic products and buffers in this kit are provided under Nuclease- and Protease-free condition. Please be careful not to contaminate any part of the kit with nucleases or proteases.

3.4. Information of components

3.4.1. Cartridges



3.4.2. Other component

A. *E. coli* extract

The *E. coli* extract in the Kit ② is a cell lysate which includes T7 RNA polymerase, ribosome, and tRNA required for protein expression. It is provided as an 8-tube strip, and each tube contains **200 μ L**.

3.5. Experimental procedure

3.5.1. Preparation of experiment

Supplies

; latex gloves, scissors, micropipettes

- ① Take out the Cartridge ② from a freezer and thaw it at room temperature. (Please ensure that all solutions are completely thawed before the experiment.)
- ② Get the Cartridge ① in the Kit ① out of a refrigerator and leave it at room temperature.
- ③ Take Disposable filter tips and a Elution tubes out of the Kit ①.
- ④ Cut off quickly 8-tube strip of *E. coli* extract as many as the number of samples and thaw them on ice.
- ⑤ When using Positive Control DNA, take out the Positive Control DNA tube from the kit② and thaw it on ice.
note) The pBIVT-AcGFP of about 3.8 kb which corresponds to 28 kDa size of protein is provided as a Positive Control DNA.
- ⑥ Prepare 1~10ug of the sample DNA. (When the sample volume is more than 10uL, take the same volume out from DEPC DW in the row H of the cartridge ②.)

Note I) The amount of sample DNA can be determined as follows.

Use 1 μ g of plasmid DNA per kb (kilobase) of that DNA, in proportional to the size of the DNA. (For example, 5 μ g of plasmid DNA is appropriate for 5 kb-sized plasmid DNA).

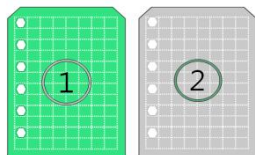
If you want to get the maximum amount of protein, please refer to the Appendix 2 for further information.

3.5.2. Protein synthesis with *ExiProgen™*

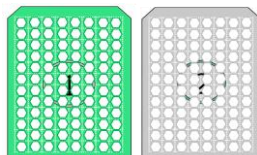
- ① Punch holes in the sealing films of Buffer Cartridge ① and ② using a 6 hole punch before installation of the Cartridges on the *ExiProgen™* instrument.

Note) The number of columns punched should be matched with the sample number.

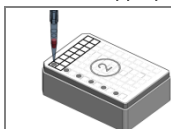
Example 1) For 1 sample



Example 2) For 16 sample



- ② Add the appropriate amount of DNA on Cartridge ②

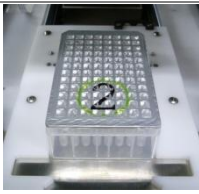
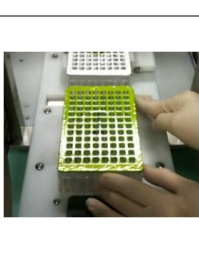
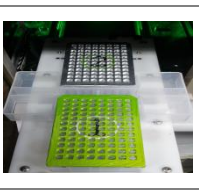
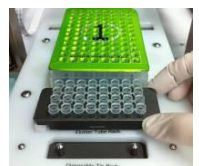


Pipette the appropriate amount of DNA in row L of Cartridge ②. (Use 10uL of Control DNA)

- ③ Install the Buffer Cartridges and related components on the *ExiProgen™* instrument as follows;



1. Open the door of *ExiProgen™* instrument, pull out the Base Plate completely.

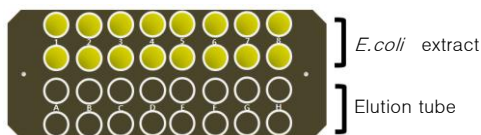
	<p>2. Install the Cartridge ② in the position of ② on the Base Plate.</p> <p>* Ensure that Cartridge ② is firmly installed without any movement and that the row L of the Cartridge ② is located on the top of the heating block of the Base Plate.</p>
	<p>3. Install the Cartridge ① in the position of ① on the Base Plate.</p> <p>* There are 'silicon rings' embedded in both sides of the installation position of Cartridge ①. Install the left side of the Cartridge ① first and then the right side. Ensure that the Cartridge ① is firmly installed, without any movement.</p>
	<p>4. Install Waste Tray between Cartridge ② and ①.</p> <p>* Follow the exact installation order of Cartridge ② → Cartridge ① → Waste Tray. Ensure that the Cartridges and the Waste Tray are firmly installed, without any movement.</p>
	<p>5. Load the Elution Tube Rack with the <i>E. coli</i> extract and Elution tubes on the prescribed position on the Base Plate.</p> <p>* Confirm that the <i>E. coli</i> extract and Elution tubes are in the correct positions (Below).</p>

<Caution>

Please refer to the illustration below when placing *E. coli* extract and Elution tube to Elution tube rack.

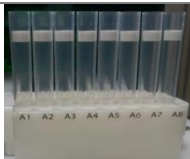
Number of Elution tube must match number of *E. coli* extract. Both must be placed on the same row as punched cartridge.

(Rows in number: *E. coli* extract, Rows in alphabet: Elution tube)



6. Place Protection cover as shown in the picture on the left.

Caution) Press down Protection cover firmly so that it covers Elution tube rack all the way down to Base plate.








7. Insert the appropriate number of Disposable filter tips into the Disposable filter tip rack and place it on the prescribed position on the Base Plate.


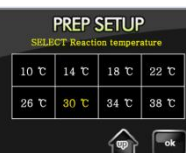






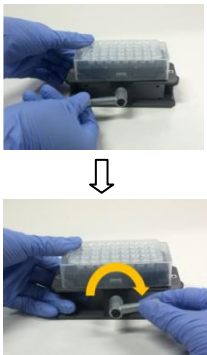
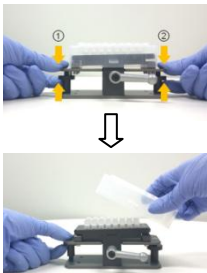
Ensure that 1) tips are located at the same columns with the punched holes of the Cartridges, and 2) there should be no tip at the columns which will not be used.

8. Push the Base Plate completely, until you hear 'click' sound or feel clicking, and close the door of *ExiProgen™*.

④ After completion of setup, run the *ExiProgen™* instrument as follows.

  	<ol style="list-style-type: none"> 1. Turn on the <i>ExiProgen™</i> instrument and press the 'Press to start' button in the center of the screen. <p>Soon the <i>ExiProgen™</i> screen with moving scroll bar appears, and after a short period of time it moves to the 'MENU' screen.</p> <p>The short pause is for the initialization process of the instrument. If it does not move to the next screen, turn off the instrument and call the customer center for A/S.</p>
	<ol style="list-style-type: none"> 2. In the MENU screen, press 'Start' button to select a proper protocol.
	<ol style="list-style-type: none"> 3. In the PREP SETUP screen shown as left, input '902' of protocol number. Ensure that the statement Prep type: Protein Sample SRC: Synthesis appears on the screen. Then select 'Enter'.

	<p>4. Choose 'ok' to go to the next step.</p> <p>* This step is for nucleic acid extraction only.</p>
	<p>5. Choose '<u>30 °C</u>' and 'ok' to go to the next step.</p>
	<p>6. Ensure that everything is correctly installed following the CHECK LIST, then choose 'ok'. If not, make an appropriate change.</p>
 	<p>7. In the Running Mode screen shown as left, ensure that the statement</p> <p>Prep type: Protein Sample SRC: Synthesis</p> <p>appears on the screen. Then press 'RUN' to initiate protein synthesis.</p> <p>'Work Completion' screen appears when the protocol is completed. Open the door and collect purified proteins. Remove all components used in the experiment, and choose 1, 2, or OK;</p> <p>* UV lamp will be on automatically if 'ok' button is selected to finish the use of the ExiProgen™.</p>

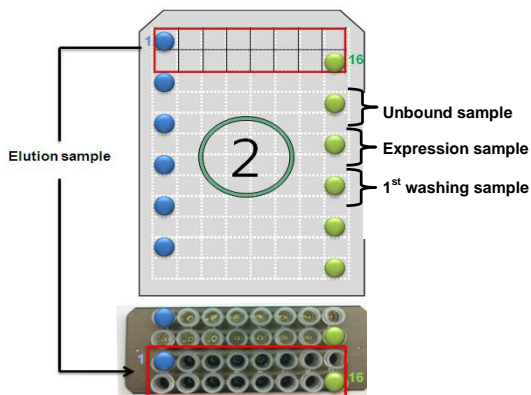
	<p>8. Please remove Protection Cover according to Protection Cover Separation Tool utility method.</p> <p>① Take out Elution Tube Rack from <i>ExiProgen™</i> and place it on top of Protection Cover Separation Tool.</p> <p>Note: When placing Elution Tube Rack on Protection Cover Separation Tool, the lever must be facing left-hand side.</p>
	<p>② Firmly hold down Protection Cover and Separation Tool with one hand. Rotate the lever in a clockwise 180° with the other hand.</p> <p>Note: Rotate the lever until Elution Tube Rack is firmly fixed to Protection Cover Separation Tool</p>
	<p>③ Press down both sides of Separation Tool as shown in the picture below. This action will push Protection Cover upwards so that Elution Tube Rack can be removed with ease.</p> <p>Tip: Hold down Protection Cover with one hand. Then press down each side of Separation Tool consecutively to prevent any liquid from splashing.</p>

3.6. Analysis of sample

After the synthesis of protein using the *ExiProgen*™ instrument, the final target protein in about 250 uL is collected from the Elution tube. (It may contain trace amount of Ni-NTA magnetic beads, which has no influence to the protein and can be easily removed through centrifugation at 10,000 rpm for 1 min).

Some of reaction samples from protein expression and/or purification processes could also be collected from the wells of row K, J, or I at the Cartridge ② as shown in Figure 3.

Figure 3. Samples at the selected rows of Cartridge ②



* **Expression sample**
; Samples after expression, but without purification

* **Unbound sample**
; Samples not bound to Ni-NTA beads

* **1st washing sample**
; Buffer used for 1st washing step in protein purification process

Through SDS-PAGE or other methods a user is able to check whether the desired protein is synthesized accordingly. Here is the protocol for SDS-PAGE:

- a. Prepare loading mixture as shown in the table.

	Elution sample	Expression/Unbound/ 1 st washing samples
sample	15 uL	5 uL
4x loading dye	5 uL	5 uL
Sterile DW	–	10 uL
Total	20 uL	20 uL

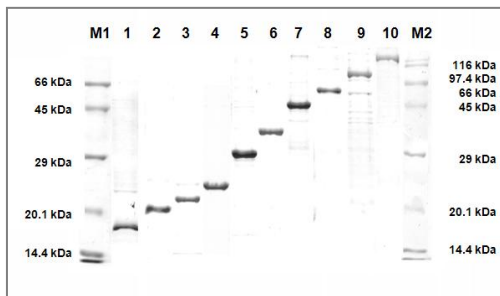
- b. Treat the samples at 95°C for 5–10 min.

- c. Prepare SDS-PAGE mini-gel either 10% or 12% SDS-PAGE gel (10x8 (cm), 10 wells) is typically used.

Load 10 uL of “Elution sample” and 5 uL each of “ Expression /Unbound/1st washing samples” to a well and run the SDS-PAGE.

- d. After staining of the gel with Coomassie blue solution, a band of target protein is detected as in Figure 4.

Figure 4. Final target protein on SDS-PAGE



M1; AccuLadder™ Protein Size Marker (Low)

1; CalmL3 (17.5kDa) , 2; RNase H (20kDa), 3; DUSP 3 (22kDa),
4; CAT (24kDa), 5; AcGFP (29kDa), 6; EF-Ts (34kDa), 7; VF (45kDa),
8; Poly A polymerase (50kDa), 9; MMLV RTase (75kDa), 10; BM3 (117kDa)

M2; AccuLadder™ Protein Size Marker (Broad)

4. Maintenance

After protein synthesis, wash and store the cartridges and other accessories as follows.

A. Waste tray and Disposable tip rack

Discard all the solution in the waste tray, wash it in running water and clean it with 20% ethanol. Also, If there is any dirty sludge on the Disposable tip rack, clean it with 20% ethanol solution. And store at room temperature.

B. Cartridge ①, ②

The Cartridges with unused wells should be covered with their lid and be stored in a refrigerator (Cartridge ①) and in a freezer (Cartridge ②).

5. Troubleshooting guide

Please refer to the troubleshooting guide below if there is a problem in protein synthesis/purification using Bioneer's *ExiProgen*™ protein synthesis kits and the *ExiProgen*™ instrument. Keep in mind those solutions in the guide are for most of proteins, but not for all proteins.

1. No protein synthesis, including positive control protein

Causes	Solutions
Contamination of nucleases (DNase or RNase)	Wear gloves all times. Use DNase- & RNase-free pipette tips.
Pipetting error or no reagent added	Check accuracy of pipettes. Add exact amount of DNA and/or reagents. Mix every reagents exactly according to the protocol.
Inappropriate storage of reagents	Store every reagents/components in the kit at recommended temperature. No repeated freeze-and-thaw of <i>E. coli</i> extract.

2. No protein synthesis, except positive control protein

Causes	Solutions
Error(s) in gene sequence of Template DNA	Check that the target gene contains start codon (ATG) and stop codon (TAA, TAG, or TAG) at the right position. Check that the target gene codon is in-frame. Check if there is a mutation in the ORF (Open Reading Frame) of template DNA (Translation may be stopped.).

Structure of expression vector	Check that the gene of a target protein is cloned into the expression vector with T7 expression system containing T7 promoter, T7 terminator, and RBS. Add appropriate amount of IPTG (Isopropyl β -D-1-thiogalactopyranoside) to the reaction mixture if the expression vector can produce endogenous lac repressor.
Contamination of template DNA	Prepare and use fresh, uncontaminated template DNA.

3. Low yield of target protein

Causes	Solutions
Low purity of template DNA	Use template DNA of which $A_{260/280}$ is between 1.7 and 2.0, and $A_{260/230}$ is over 1.5.
Concentration of template DNA	Determine and use the optimal concentration of template DNA (see Appendix 2.).
No codon optimization of template DNA to <i>E. coli</i>	Do the codon optimization of target gene to <i>E. coli</i> .
Location of histidine tag	Try to locate the histidine tag on the different location of target protein. * Histidine tag may inhibit expression of target protein due to its location. The expressed target protein may not be purified through Ni-NTA beads, if histidine tag is not exposed outside effectively.

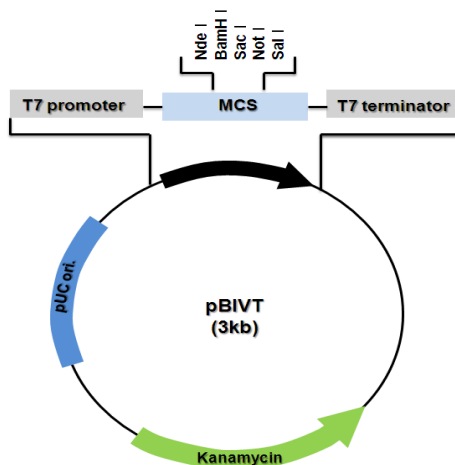
4. Low activity and/or solubility of target protein

Causes	Solutions
Requiring posttranslational modification for activity and/or solubility	Not available yet, due to the lack of posttranslational modification in cell-free protein synthesis using <i>E. coli</i>
Requiring cofactor for protein activity	Add the cofactor required to the final target protein.
Aggregation of protein due to low solubility	Synthesize protein at lower temperature. Add chaperone protein to help protein folding.

6. Appendix 1 : pBIVT vectors

pBIVT vector set (Cat. No. K-7350) is designed for Bioneer's cell-free protein synthesis system. It fits well into all kits of ExiProgen™ protein synthesis system.

Using this vector set it is possible to add 6x histidine tag to either N-terminal or C-terminal of target protein. The structure of vector is as follows.



① pBIVT-1's MCS (Multi Cloning Site) sequences

CATATGCACCACCACCACCACGGATCCGAGCTCAAGCTTGC

Nde I 6x histidine tag BamH I Sac I Not I
ATAGGTCGAC
 Sal I

② pBIVT-2's MCS (Multi Cloning Site) sequences

CATATGGGATCCGAGCTCAAGCTTGGGCGGCACACCACCACCACC

Nde I BamH I Sac I Not I 6x histidine tag
ACTAGGTCGAC
 Sal I

7. Appendix 2

: Determination of template DNA concentration

A different amount of template DNA in protein synthesis reaction with *ExiProgen™* may result in a different yield of target protein. It is strongly recommended to optimize the amount (or concentration) of template DNA for protein synthesis in the *ExiProgen™*.

For the optimization of template DNA amount it is useful to use the *AccuRapid™* Cell-free Protein Expression Kit (Cat. No. K-7250). Protocol is shown in the below.

AccuRapid™ Cell-Free Protein Expression Kit (Cat. No. K-7250)

1. Prepare template DNA with the following concentrations.
Ex) 25, 50, 100, 200, 300, and 400 (ng/μL)
2. Express the target protein following the manual of *AccuRapid™* Cell-free Protein Expression Kit
 - 1) Prepare reaction mixture
 - Reaction volume ; 45 μL
 - Sample DNA
 - ; Add 2 μL of template DNA from #1.
 - ; Final DNA amount
 - Ex) 50, 100, 200, 400, 600, and 800 ng/rxn
 - 2) Incubate the mixture at 30 °C for 3 hrs.
 - 3) Verify the amount of template DNA which shows the highest yield of target protein, through SDS-PAGE.



ExiProgen™ EC Protein Synthesis Kit

3. Determine the 16.7-times as much DNA as the optimized amount from 2.
4. Add the determined amount of template DNA from 3. Then synthesize protein using the *ExiProgen™* EC Protein Synthesis Kit.

8. References

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9. Related products

Cat. No.	Product	Size
K-7250	<i>AccuRapid™</i> Cell-Free Protein Expression Kit	45 uL x 24 reactions
K-7260	<i>AccuRapid™</i> Midi Protein Expression Kit	1 mL x 5 reactions
K-7270	<i>AccuRapid™</i> Maxi Protein Expression Kit	10 mL x 1 reaction
K-7310	<i>ExiProgen™</i> EC-Maxi Protein Synthesis Kit	8 reactions
K-7320	<i>ExiProgen™</i> EC-Tagfree Protein Synthesis Kit	8 reactions
K-7330	<i>ExiProgen™</i> EC-Disulfide Protein Synthesis Kit	8 reactions
K-7350	pBIVT vector set	Each 5ug
K-7400	<i>ExiProgen™</i> ProXpress PCR Template Kit	16 reactions
K-7401		32 reactions
D-2010	<i>AccuLadder™</i> Protein Size Marker (Broad)	500 uL
D-2020	<i>AccuLadder™</i> Protein Size Marker (Low)	500 uL
S-2041	Gene Synthesis Service	–
S-2500	Protein Synthesis Service	–
A-5041	<i>ExiProgen™</i>	–