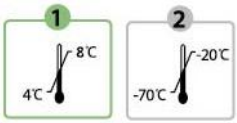
User's Guide

ExiProgen™
EC-Maxi Protein Synthesis Kit

REF K-7310



BIONEER
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ExiProgen™ EC-Maxi Protein Synthesis Kit

User's Guide

Version No.: 2.0 (2016-02)

Please read all the information in booklet before using the kit

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Safety warning and Precaution

ExiProgen™ EC-Maxi 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, and KR 10-2012-0138335.

Trademark

ExiProgen™ is trademark of Bioneer Corporation.

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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.

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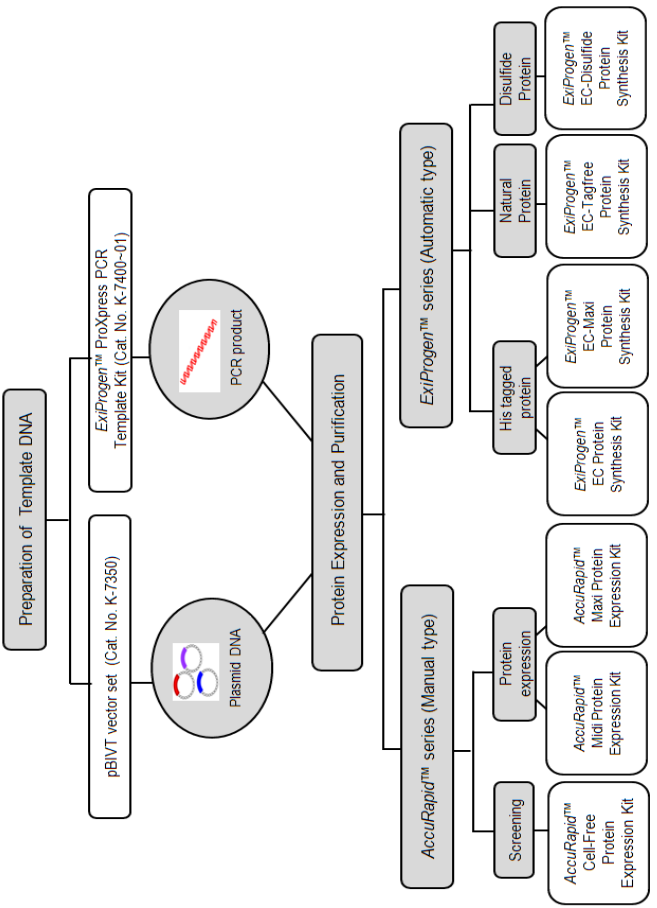
2. The *ExiProgen*™ protein synthesis system

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.

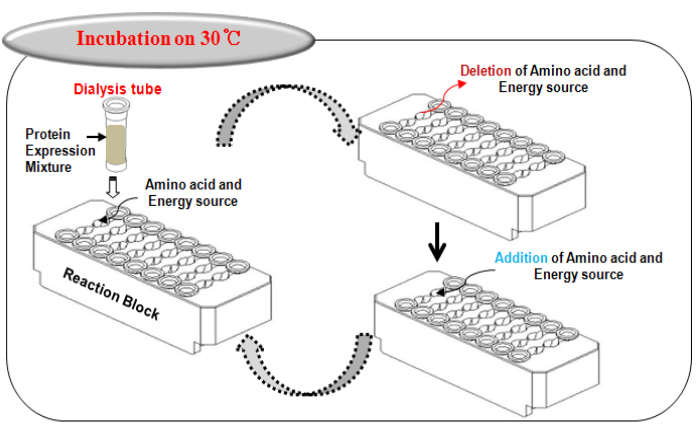

Preparation of Template DNA
pBIVT vector set (Cat. No. K-7350)
ExiProgen™ ProXpress PCR Template Kit (Cat. No. K-7400~01)
Plasmid DNA
PCR product
Protein Expression and Purification
AccuRapid™ series (Manual type)
ExiProgen™ series (Automatic type)
Screening
Protein expression
His tagged protein
Natural Protein
Disulfide Protein
AccuRapid™ Cell-Free Protein Expression Kit
AccuRapid™ Midi Protein Expression Kit
AccuRapid™ Maxi Protein Expression Kit
ExiProgen™ EC Protein Synthesis Kit
ExiProgen™ EC-Maxi Protein Synthesis Kit
ExiProgen™ EC-Tagfree Protein Synthesis Kit
ExiProgen™ EC-Disulfide Protein Synthesis Kit

3. *ExiProgen*™ EC-Maxi Protein Synthesis Kit

3.1. Overview

ExiProgen™ EC-Maxi Protein Synthesis Kit generates protein automatically through Bioneer's *ExiProgen*™ instrument, up to 500 μg per reaction of protein with high purity. All the processes of protein expression, purification and dialysis of target proteins are completed in one day and the final target protein is collected in storage buffer.

This kit shares the same principle of cell-free protein expression and Ni-NTA magnetic bead-based protein purification with the *ExiProgen*™ EC Protein Synthesis Kit. But one notable feature in the *ExiProgen*™ EC-Maxi Protein Synthesis Kit is a higher protein yield, which is accomplished through continuous supply of energy source required for protein expression. The principle is shown below.



3.2. Prepare of template DNA

A protein expression vector can be used as template DNA for the *ExiProgen*™ EC-Maxi Protein Synthesis Kit. Figure 1 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 1. 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 1of 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-Maxi Protein Synthesis Kit ①		
Cartridge ①	96 well x 1ea	Store at 4 ~ 8℃
Dialysis tube	1 pack (16 ea/pack)	
Disposable filter tip	1 pack (8 ea/pack)	Store at Room temperature
Protection cover	1 ea	

ExiProgen™ EC-Maxi Protein Synthesis Kit ②		
Cartridge ②	96 well x 1ea	Store at −20 ~ −70℃
E. coli extract	8-tube strip (Yellow) x 1 ea	
Master mix	8-tube strip (Violet) x 1 ea	
DEPC DW	8-tube strip (White) x 1 ea	
Storage buffer	35 mL x 2 bottle	
Positive Control DNA	1.5mL tube x 1 ea	

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

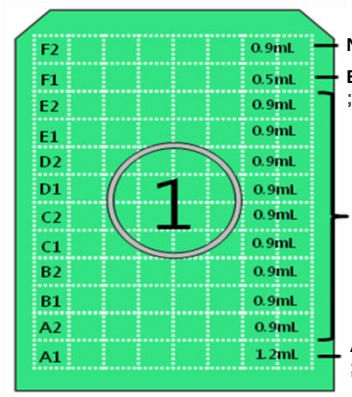
The Kit ① includes Cartridge ①, Disposable filter tip, Dialysis tube, and a Protection cover. Cartridge ① contains buffers to be used for protein purification and should be stored in a refrigerator (4-8°C) along with Dialysis tube.

The Kit ② includes Cartridge ②, Master mix, DEPC DW, *E. coli* extract, Storage buffer and a tube of Positive Control DNA. Cartridge ② contains Feeding solution 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



Ni-NTA magnetic bead

Elution buffer
; Buffer for purification of target protein (1M imidazole included)

Binding/Washing buffer
; Buffer for binding of expressed proteins on Ni-NTA magnetic beads & for washing process to remove impurities

Autoclaved DW
; Distilled Water for tip

Feeding buffer
; Buffer for protein expression to be added to the reaction mixture. It contains NTPs, amino acids, and energy source etc.

Storage buffer
; Buffer for removal of imidazole and storage of final target protein

3.4.2. Other components

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 **130 uL**.

B. Master mix

The Master mix, which is also in the Kit ②, includes NTPs, amino acids, energy sources and salts required for protein expression. It is provided as an 8-tube strip, and each tube contains **220 uL**.

C. DEPC DW

It is used to adjust final volume of protein expression solution. It is provided as an 8-tube strip, and each tube contains **130 uL**.

D. Dialysis tube

Dialysis tube, which is specifically manufactured by Bioneer, is used to supply the protein source materials from the Feeding buffer to the protein expression solution, and to exchange the purification buffer to Storage buffer after purification of a target protein. It is provided in 20% ethanol and individually wrapped can be used as many as needed. It is required to remove 20% ethanol and rinse out with distilled water once before use.

E. Storage buffer

Composition of Storage buffer in the Kit ② is as follows;

50mM Tris-Cl (pH7.6), 100mM NaCl, 1mM DTT, 0.1mM EDTA, 0.05%(v/v) NaN_3, 50%(v/v) glycerol

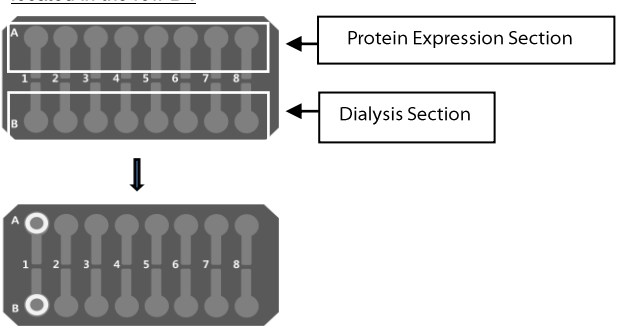
Please add 1 ml of the storage buffer provided in two separate bottles in the kit to each well of G1 to J1 in Cartridge② right before the use. When necessary, a user may construct another buffer with different composition and use it instead of the storage buffer provided by the manufacturer. Just keep in mind that a user's own buffer should contain 10% of glycerol not to overflow out of the Reaction block.

3.5. Experimental procedure

3.5.1. Preparation of experiment

Supplies
; latex gloves, forceps, sterile distilled water in squeeze bottle , scissors, micropipettes, Reaction block (provided with the *ExiProgen*™ instrument)

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 Prepare the Reaction block.
④ Take out twice as many Dialysis tubes as the number of samples, remove the 20% ethanol solution and rinse out the inside and the outside of the tube with sterile distilled water.
⑤ Install the DW-rinsed Dialysis tube on a Reaction Block as the diagram below indicates. Fill 500 uL of sterile distilled water in the Dialysis tube located in the row B .



⑥ Take Disposable filter tips and a Protection cover out of the Kit ①.

3.5.2. Preparation of protein expression mixture

The first step of protein synthesis using the *ExiProgen*™ is to prepare the protein expression solution containing template DNA.

① Cut off quickly 8-tube strip of *E. coli* extract, Master mix, and DEPC DW as many as the number of samples and thaw them on ice.
② 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.
③ Prepare protein expression solution using pre-thawed *E. coli* extract, Master mix and DEPC DW. It is neccessary to spin-down each tube with a microcentrifuge and then mix with a pipette before use.

	Positive Control	Sample
Template DNA	6 uL	X uL
E. coli extract	120 uL	120 uL
Master mix	210 uL	210 uL
DEPC DW	114 uL	(120-X) uL
Total	450 uL	450 uL

Note) The amount of template DNA, in microgram quantity, can be determined as follows.
Use 0.6 μg of plasmid DNA per kb (kilobase) of that DNA, in proportional to the size of the DNA. (For example, 3 μg 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.

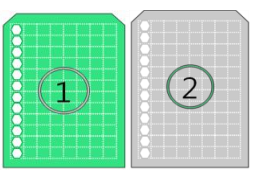
④ Remove water completely from the inside of Dialysis tube in the row A of the reaction block, then add protein expression solution.
⑤ Cover the Protection cover on a Reaction Block.

3.5.3. Protein synthesis with *ExiProgen*™

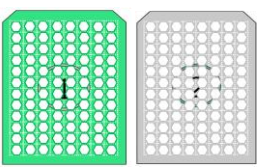
① Punch holes in the sealing films of Buffer Cartridge ① and ② using a 6 hole punch before installation of the Cartridges on the *ExiProgen*™ instrument, and add the Storage buffer on G1~J1 rows of Cartridge ② (Refer to the page 8~9).

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

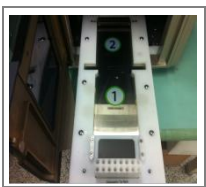
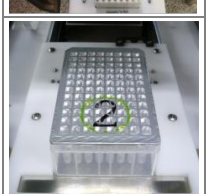
Example1) For 1 sample

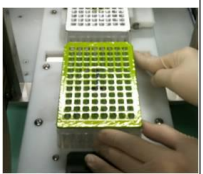
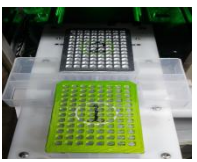
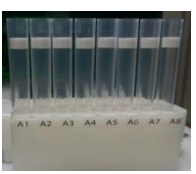


Example 2) For 8 sample

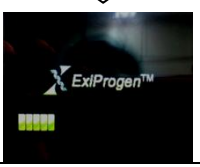


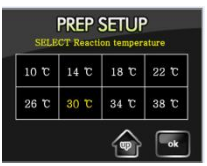
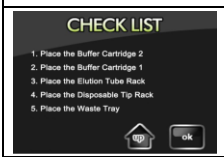
② Install the Buffer Cartridges and related components on the *ExiProgen*™ instrument as follows;

	1. Open the door of *ExiProgen*™ instrument, pull out the Base Plate completely.
	2. Install the Cartridge ② in the position of ② on the Base Plate. * 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.

	3. Install the Cartridge ① in the position of ① on the Base Plate. * 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
	4. Install Waste Tray between Cartridge ② and ①. * 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.
	5. Install the Reaction Block on the magnetic part of the Base Plate (see the left figure). * Ensure that the row A of the Reaction block is located toward the two Cartridges (inside) and the row B toward a user (outside).
	6. Load as many Disposable tip(s) as the number of samples into the same location of column with punched hole(s) in the **row B of the Disposable Tip Rack** * 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.
	7. Push the Base Plate completely, until you hear 'click' sound or feel clicking, and close the door of *ExiProgen*™.

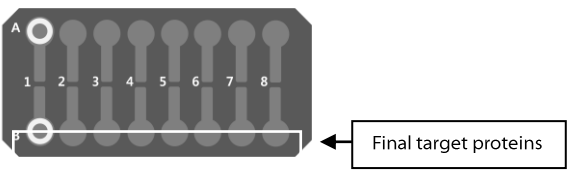
③ After completion of setup, run the *ExiProgen*™ instrument as follows.

 ⇩ 	1. Turn on the *ExiProgen*™ instrument and press the 'Press to start' button in the center of the screen. Soon the *ExiProgen*™ screen with moving scroll bar appears, and after a short period of time it moves to the 'MENU' screen. 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.
	2. In the MENU screen, press 'Start' button to select a proper protocol.
	3. In the PREP SETUP screen shown as left, input '903' of protocol number. Ensure that the statement **Prep type: Protein** **Sample SRC; Synthesis_Maxi** appears on the screen. Then select 'Enter'.

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

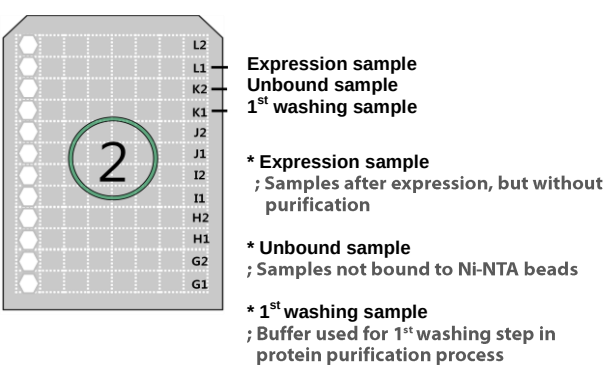
3.5.4. Analysis of sample

After the synthesis of protein using the *ExiProgen*™ instrument, the final target protein in about 220~230 uL of Storage buffer is collected from the Dialysis tube in the row B of Reaction Block. (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 L1, K1, or K2 at the Cartridge ② as shown in Figure 2.

Figure 2. Samples at the selected rows of Cartridge ②



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.

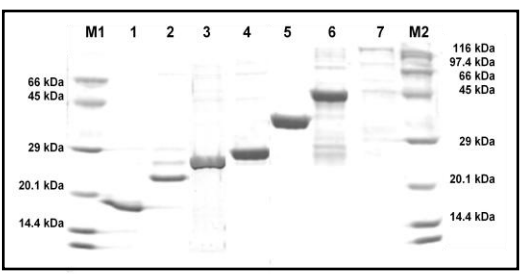
	Final target protein solution	Expression/Unbound/ 1st washing samples
sample	5 uL	5 uL
4x loading dye	5 uL	5 uL
Sterile DW	10 uL	10 uL
Total	20 uL	20 uL

b. Treat the samples at 95℃ 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 "Final target protein solution" 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 3.

Figure 3. Final target protein on SDS-PAGE



M1; *AccuLadder*™ Protein Size Marker (Low), M2 ; *AccuLadder*™ Protein Size Marker (Broad), 1; CalmL3 (17.5kDa) 2; DUSP 3 (22kDa), 3; CAT (24kDa), 4; AcGFP (29kDa), 5; EF-Ts (34kDa), 6; VF (45kDa), 7; BM3 (117kDa)

4. Maintenance

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

A. Reaction Block

Rinse all the used wells of the Reaction Block with sterile distilled water. Remove any residual water completely and store it in 70% ethanol solution.

B. 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.

C. 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 *E. coli* 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

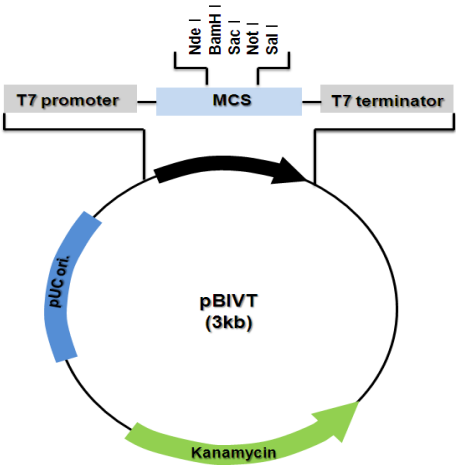
Causes	Solutions
Low purity of template DNA	Use template DNA of which $A_{260/280}$ is between 1.7 and 2.0, and $A_{260/230}$ is over 1.5.
Concentration of template DNA	Determine and use the optimal concentration of template DNA (see Appendix 2.).
No codon optimization of template DNA to *E. coli*	Do the codon optimization of target gene to *E. coli*.
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 *E. coli*
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

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

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

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

ExiProgen™ EC-Maxi Protein Synthesis Kit

3. Determine the 10-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-Maxi Protein Synthesis Kit.

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9. Related products

Cat. No.	Product	Size
K-7250	*AccuRapid*™ Cell-Free Protein Expression Kit	45 uL x 24 reactions
K-7260	*AccuRapid*™ Midi Protein Expression Kit	1mL x 5 reactions
K-7270	*AccuRapid*™ Maxi Protein Expression Kit	10mL x 1 reaction
K-7300	*ExiProgen*™ EC Protein Synthesis Kit	16 reactions
K-7301		32 reactions
K-7302		96 reactions
K-7320	*ExiProgen*™ EC-Tagfree Protein Synthesis Kit	8 reactions
K-7330	*ExiProgen*™ EC-Disulfide Protein Synthesis Kit	8 reactions
K-7350	pBIVT vector set	Each 5ug
K-7400	*ExiProgen*™ ProXpress PCR Template Kit	16 reactions
K-7401		32 reactions
D-2010	*AccuLadder*™ Protein Size Marker (Broad)	500 uL
D-2020	*AccuLadder*™ Protein Size Marker (Low)	500 uL
S-2041	Gene Synthesis Service	-
S-2500	Protein Synthesis Service	-
A-5041	*ExiProgen*™	-

ExiProgen™ EC-Maxi Protein Synthesis Kit

사 용 설 명 서

Version No.: 1.0 (2013-05)

Please read all the information in booklet before using the kit

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Safety warning and Precaution

ExiProgen™ EC-Maxi 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, and KR 10-2012-0138335.

Trademark

ExiProgen™ is trademark of Bioneer Corporation.

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1. Introduction

단백질은 효소, 호르몬, 구조단백질 등의 다양한 기능으로 생체 반응에 있어서 필수적인 요소이기 때문에, 포스트게놈시대에 이러한 단백질들의 역할 및 구조 등에 대한 연구가 활발하게 진행되고 있으며, 이러한 연구는 특정 단백질을 만들어내는 것으로부터 시작됩니다.

재조합 단백질을 합성하는 방법은 대장균, 효모, 동물세포 등 다양한 세포주를 이용하며, 재조합 단백질을 발현할 수 있는 벡터를 세포 내로 형질전환 시키고 세포주를 배양하여 재조합 단백질을 발현시킨 후 이 세포를 파쇄하여 발현된 단백질을 정제하는 방법들이 일반적으로 사용되고 있습니다. 이 방법은 재조합 단백질을 안정하게 발현시키는 균주 선별 과정을 필요로 하며, 이후 세포배양, 세포파쇄, 그리고 단백질 정제의 일련의 과정을 거쳐야 하므로 많은 시간과 노동력이 필요합니다. 특히 세포주에 독성을 나타내는 단백질인 경우에는 단백질을 발현시키는 것이 어려워 다양한 발현조건들을 시도해야만 하기에 많은 노력이 필요하여 하나의 순수한 단백질을 합성하기까지 최소 수 일에서 길게는 수개월의 시간이 소요됩니다.

이러한 문제점을 극복하고자 짧은 시간 내에 반응용기 내에서 단백질을 합성하는 무세포 단백질 발현 방법과 이와 관련한 제품들이 개발되어 왔습니다. 이 방법은 반응용기에 단백질을 발현할 수 있는 주형 DNA (예)expression vector, PCR product), 세포 파쇄액, 아미노산과 핵산 그리고 에너지물질이 들어 있는 단백질발현용액을 첨가한 후, 적정온도에서 반응을 시켜 재조합 단백질을 발현시키는 방법으로, 세포를 사용하여 단백질을 발현시키는 방법에 비해서 소요시간을 획기적으로 줄일 수 있을 뿐만 아니라 세포 내에서 독성을 가지는 단백질도 발현을 시킬 수 있다는 장점을 가지고 있습니다.

무세포 단백질 발현법은 새로운 유전자의 기능에 대한 발견뿐만 아니라 다양한 mutants 단백질들에 대한 성능을 비교하는데 사용되고 있으며, 단백질-단백질 상호작용 기작 연구, 단백질의 활성부위에 대한 연구 등에도 폭넓게 활용되고 있습니다.

2. The *ExiProgen*™ protein synthesis system

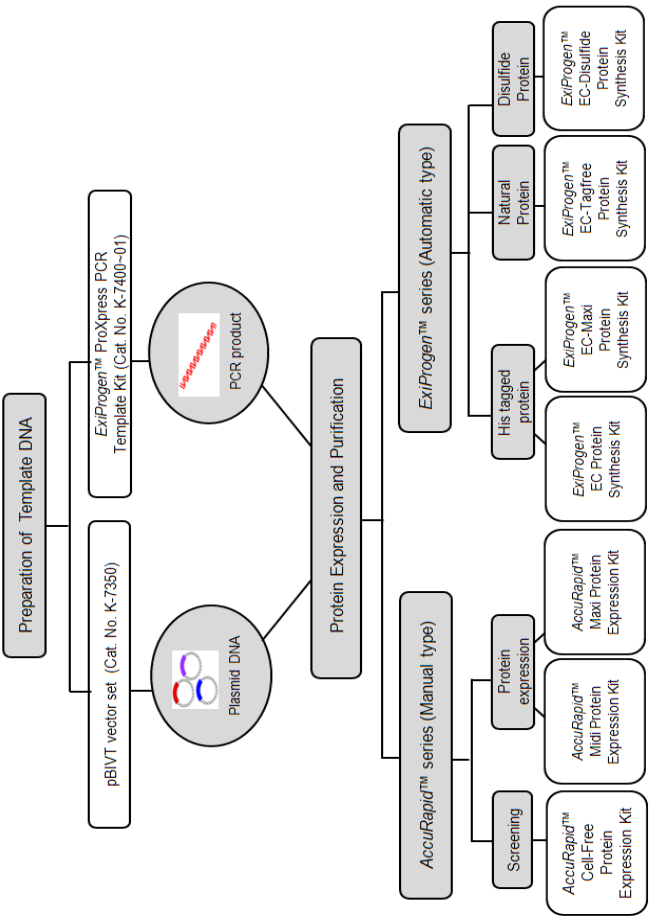
㈜바이오니아에서는 무세포 단백질 발현 방법과 나노 자성입자를 이용한 친화성 정제 방법을 이용하여 전자동으로 다양한 단백질을 합성하고 고순도로 정제할 수 있는 *ExiProgen*™ 전자동 단백질 합성 장비를 개발 했습니다.

ExiProgen™ 전자동 단백질 합성 장비는 당사의 다양한 단백질 발현 및 정제 키트를 이용하여 고순도의 단백질을 전자동으로 획득할 수 있으며, 뿐만 아니라 핵산 추출키트를 이용하여 다양한 시료로부터 필요한 DNA, RNA 를 전자동으로 추출할 수 있습니다.

당사의 단백질 발현 및 합성 키트는 세 가지 제품 군(33 페이지 참조)으로 분류됩니다. 무세포 단백질 발현에 사용되는 template DNA 를 제조하는 키트와 Manual 방식으로 손쉽게 재조합 단백질 발현할 수 있는 *AccuRapid*™ series 및 *ExiProgen*™을 이용하여 전자동으로 단백질을 발현하고 정제할 수 있는 *ExiProgen*™ series 로 나누어 집니다.

당사의 키트는 *E. coli* 의 T7 발현 시스템을 이용하며, 주형 DNA (Expression vector 또는 PCR product)만 가지고 있으면 곧바로 단백질 발현에 사용할 수 있습니다. 또한 *ExiProgen*™ 장비를 이용한 전자동 단백질 합성은 주형 DNA 를 이용하여 단백질 발현한 후, 목적 단백질을 정제하는 순차적인 반응으로 진행이 됩니다.

각 키트를 이용하여 최소 ug 단위부터 최대 mg 단위까지 단백질의 발현 및 합성이 가능합니다. 각 키트에 대한 자세한 설명은 바이오니아 홈페이지 (www.bioneer.co.kr)을 참조하시기 바랍니다.

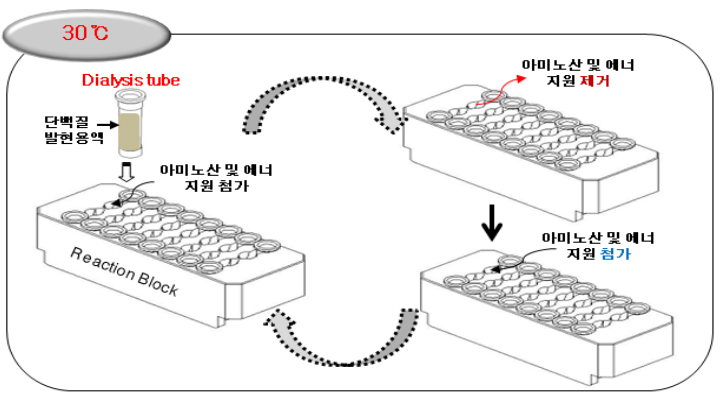

Preparation of Template DNA
pBIVT vector set (Cat. No. K-7350)
ExiProgen™ ProXpress PCR Template Kit (Cat. No. K-7400-01)
Plasmid DNA
PCR product
Protein Expression and Purification
AccuRapid™ series (Manual type)
Screening
Protein expression
AccuRapid™ Cell-Free Protein Expression Kit
AccuRapid™ Midi Protein Expression Kit
AccuRapid™ Maxi Protein Expression Kit
ExiProgen™ series (Automatic type)
His tagged protein
Natural Protein
Disulfide Protein
ExiProgen™ EC Protein Synthesis Kit
ExiProgen™ EC-Maxi Protein Synthesis Kit
ExiProgen™ EC-Tagfree Protein Synthesis Kit
ExiProgen™ EC-Disulfide Protein Synthesis Kit

3. *ExiProgen*™ EC-Maxi Protein Synthesis Kit

3.1. Overview

ExiProgen™ EC-Maxi Protein Synthesis Kit는 당사의 *ExiProgen*™ 장비를 이용하여 전자동으로 단백질을 합성할 수 있는 키트이며, 1회 반응당 순도 높은 단백질을 최대 500 ug까지 합성할 수 있습니다. 또한 단백질 발현/정제 및 투석까지 모든 반응이 하루에 전자동으로 이루어지며, 최종 목적 단백질은 Storage buffer에 용해된 상태로 회수할 수 있습니다.

이 키트는 당사의 *ExiProgen*™ EC Protein Synthesis Kit와 동일한 원리인 무세포 단백질 발현법을 이용하여 단백질을 발현하고, 발현된 단백질을 Ni-NTA magnetic bead를 이용하여 정제하는 키트입니다. 그러나 기존 키트와 달리 단백질 발현에 필요한 에너지원 등을 지속적으로 공급해줌으로써, 단백질 발현 수율을 높인 키트이며, 그 원리는 아래와 같습니다.



3.2. Prepare of template DNA

ExiProgen™ EC-Maxi Protein Synthesis Kit 의 template DNA 로 발현 벡터를 사용할 수 있습니다, template DNA 는 " T7 promoter – Ribosome binding site (RBS) – Target gene – T7 terminator"의 구조를 가지고 있어야 하며, 그 구조는 그림 1 과 같습니다. 또한 target gene 은 start codon 과 stop codon 을 가지고 있어야 하며, 정제를 위하여 5'말단 또는 3'말단에 6x histidine tag 을 가지고 있어야 합니다.

그림 1. Template DNA 의 구조

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

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

발현 벡터는 ㈜바이오니아 에서 판매하는 *In vitro* translation 전용 벡터인 pBIVT 벡터 (Cat. No. K-7350, Appendix 1. 참조)에 클로닝하여 사용 가능하며, 그 밖에도 pK7, pIVEX, pET 벡터 등을 사용할 수 있습니다.

단백질 합성을 최대화 하기 위해서는 DNA 의 서열이 *E. coli* 코돈에 최적화되어 있어야 하며, 만약 코돈 최적화가 안되어 있는 경우 당사의 Gene synthesis service 를 통해서 합성하실 수 있습니다. 또한 당사의 *In vitro* translation 전용 vector 에 클로닝 된 상태로 제공 받으실 수 있습니다. 자세한 내용은 홈페이지 (www.bioneer. co.kr)를 참조하시기 바랍니다.

3.3. Kit contents and storage

ExiProgen™ EC-Maxi Protein Synthesis Kit ①		
Cartridge ①	96 well x 1ea	Store at 4 ~ 8℃
Dialysis tube	1 pack (16 ea/pack)	
Disposable filter tip	1 pack (8 ea/pack)	Store at Room temperature
Protection cover	1 ea	

ExiProgen™ EC-Maxi Protein Synthesis Kit ②		
Cartridge ②	96 well x 1ea	Store at −20 ~ −70℃
E. coli extract	8-tube strip (Yellow) x 1 ea	
Master mix	8-tube strip (Violet) x 1 ea	
DEPC DW	8-tube strip (White) x 1 ea	
Storage buffer	35 mL x 2 bottle	
Positive Control DNA	1.5mL tube x 1 ea	

ExiProgen™ EC-Maxi Protein Synthesis Kit 는 Kit ①과 Kit ②로 구성되어 있습니다.

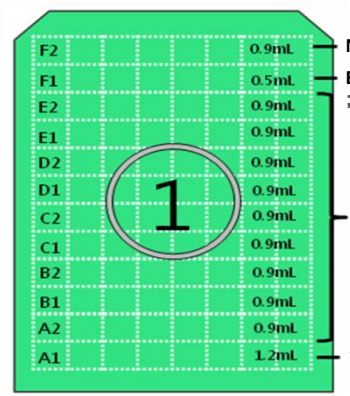
Kit ①에는 단백질 정제에 사용할 buffer류를 포함하는 Cartridge ①과 Disposable filter tip, Dialysis tube 그리고 Protection cover가 포함되어 있으며, 이 중 Cartridge ①은 냉장보관 (4~8℃) 해야 합니다.

또한 Kit ②는 단백질 발현에 사용할 Feeding buffer가 들어있는 Cartridge ②와 Master mix, *E. coli* extract, DEPC DW, Positive Control DNA 및 Storage buffer가 포함되어 있으며, 냉동보관 (−20~−70℃)에서 보관해야 합니다. (이 중 *E. coli* extract는 deep freezer (−70℃)에서 보관하는 것을 권장합니다.)

본 키트의 Cartridges는 교차 오염, 증발, 용액 누출을 막기 위해 Sealing film으로 밀봉 포장 되어 있고, 키트의 모든 플라스틱 제품이나 버퍼 류는 DNase-free, RNase-free 상태로 제공되므로 보관 및 사용 중 Nuclease 또는 Protease에 의해서 오염되지 않도록 주의 하시기 바랍니다.

3.4. Information of components

3.4.1. Cartridges

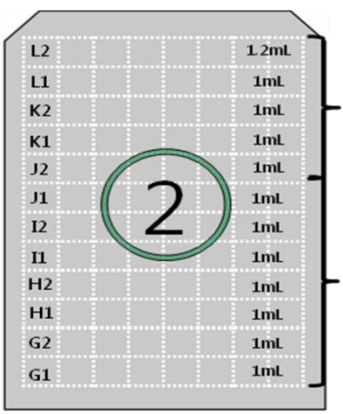


Ni-NTA magnetic bead

Elution buffer
; 목적 단백질 정제용 buffer
(1M imidazole을 포함하고 있음)

Binding/Washing buffer
; Ni-NTA magnetic bead에 단백질 발현물이 결합할 수 있게 평형화 해주며, 목적 단백질 결합 후, 세척과정 (불순물제거)에 사용

Autoclaved DW
; Elution 전, tip 세척에 사용



Feeding buffer
; 순차적 단백질 발현 반응시, 첨가해 주는 단백질 발현용액으로, NTP, 아미노산, 에너지원 등을 포함하고 있음

Storage buffer
; 단백질 정제 후, imidazole을 제거해 주고 최종 목적 단백질을 보관해주는 완충용액

3.4.2. Other components

A. *E. coli* extract

Kit ②의 구성품인 *E. coli* extract는 세포파쇄액으로, 단백질 발현에 필요한 필요한 T7 RNA polymerase, Ribosome, tRNA 등을 공급해 주며, 8-tube strip으로 공급되며, 각 tube당 **130 uL**씩 분주되어 있습니다.

B. Master mix

Kit ②의 구성품인 Master mix는 단백질 발현에 필요한 NTPs, 아미노산 및 에너지원과 salt를 포함하고 있으며, 8-tube strip으로 공급되며, 각 tube당 **220 uL**씩 분주되어 있습니다.

C. DEPC DW

단백질 발현용액의 최종 부피를 맞추어 주는 용도로 사용되며, 8-tube strip으로 공급되며, 각 tube당 **130 uL**씩 분주되어 있습니다.

D. Dialysis tube

Dialysis tube 는 당사에서 제작한 특수한 membrane tube 로, Feeding buffer 내의 에너지원을 단백질발현용액으로 공급해 주고, 목적 단백질 정제 후 정제버퍼를 Storage buffer 로 교체할 때 사용되며, 20% ethanol 에 담겨져 있습니다. 또한 각 tube 는 개별 포장되어 있으므로, 사용시 필요한 개수만큼 꺼내어 사용하시면 됩니다. 사용하기 전 20% 에탄올을 제거하고 DW 로 한번 세척한 후 사용하기 바랍니다.

E. Storage buffer

키트에서 제공하는 Storage buffer 의 조성은 다음과 같습니다.

50mM Tris-Cl (pH7.6), 100mM NaCl, 1mM DTT, 0.1mM EDTA, 0.05%(v/v) NaN_3, 50%(v/v) glycerol

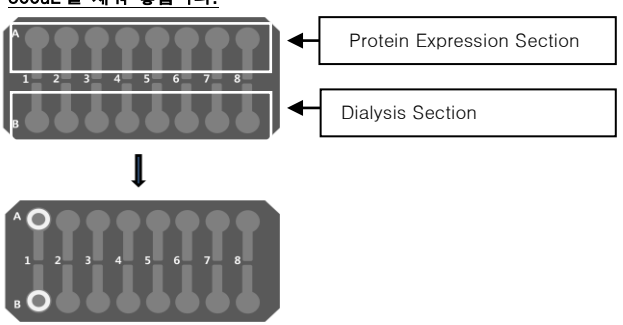
Storage buffer 는 bottle 에 제공되며, 사용 직전에 Cartridge ②의 G1~J1 열에 1mL 씩 분주하여 사용하시면 됩니다. 만일 다른 조성의 buffer 를 사용하시고자 하는 경우 조성에 맞게 직접 제조하여 사용하시면 됩니다. 단, buffer 에 glycerol 을 10% 이상 첨가해 주셔야 합니다. 그렇지 않을 시, 투석 튜브내의 용액이 Reaction block 밖으로 넘쳐 흐를 수 있습니다.

3.5. Experimental Procedure

3.5.1. Preparation of experiment

준비물 ; 라텍스 장갑, 핀셋, 멸균수가 들어있는 squeeze bottle,
가위, micropipettes, Reaction block (*ExiProgen*™ 악세사리)

① 냉동보관 중인 Cartridge ②를 상온에서 미리 녹입니다.
(사용 전 모든 용액이 완전히 녹은 것을 확인하고 사용하시기 바랍니다.
참고) 상온(20~25℃의 경우 약 2 시간 소요됨)
② 냉장보관중인 Cartridge ①은 꺼내서 상온에 둡니다.
③ Reaction block 을 준비합니다.
④ 키트 ① 박스에서 Dialysis tube 를 sample 수의 2 배의 개수로 꺼낸다.
핀셋으로 tube 를 꺼낸 후, pipettes 을 이용하거나, 상하로 흔들어 주어 tube 내의 에탄올을 제거합니다. 그 후, squeeze bottle 에 있는 멸균증류수로, tube 안과, 바깥쪽을 세척해줍니다.
⑤ Pipettes 을 이용하여 tube 내의 물기를 완전히 제거하고, Reaction block 에 하기 그림과 같이 장착하고, **B 행의 tube 에는 멸균증류수 500uL 를 채워 놓습니다.**



⑥ 키트 ① 박스에서 Disposable filter tip 그리고 Protection cover 를 꺼내서 준비합니다.

3.5.2. Preparation of protein expression mixture

ExiProgen™으로 단백질 합성을 하기에 앞서, 발현시키고자 하는 DNA 가 첨가된 발현 용액을 제조해야 합니다.

① 키트 ②번 박스에서 *E. coli* extract, Master mix, DEPC DW tube 를 반응시료의 수에 맞게 꺼내어 녹입니다.
② (Positive Control DNA 를 사용하고자 하는 경우)
키트 ②번 박스에서 Positive Control DNA tube 를 꺼내어 녹입니다.
참고) Positive Control DNA 로는 pBIVT-AcGFP 가 제공되며, 약 3.8kb (단백질 크기 28kDa 의 분자량)입니다.
③ 미리 녹인 *E. coli* extract 와 Master mix, DEPC DW 를 이용하여 하기와 같이 단백질 발현용액을 제조합니다. (각 tube 를 spin-down 한 후, tube 내의 용액을 pipette 으로 mixing 한 후 사용하시기 바랍니다.)

	Positive Control	Sample
Template DNA	6 uL	X uL
E. coli extract	120 uL	120 uL
Master mix	210 uL	210 uL
DEPC DW	114 uL	(120-X) uL
Total	450 uL	450 uL

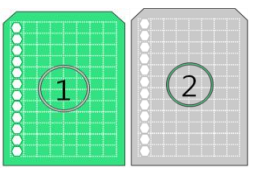
참고) Sample DNA 의 양은 다음과 같이 정할 수 있습니다.
일반적으로 plasmid DNA 크기에 비례하여 0.6ug/kb (ex) plasmid DNA ; 5kb → 3 ug 사용)를 첨가하시면 되지만, 최적의 단백질 합성을 원하시는 경우 Appendix 2.를 참조하여 DNA 농도 스크리닝 실험을 선행하시기 바랍니다.

④ 위에서 제조한 각 단백질 발현 용액을 Reaction block 의 **A 행의 tube**에 넣습니다.
⑤ Reaction block 위에 Protection cover를 씌워 실험 준비를 마칩니다.

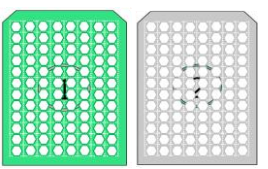
3.5.3. Protein synthesis with *ExiProgen*™

① *ExiProgen*™ 장비에 장착하기에 앞서, 6 Hole-punch (*ExiProgen*™ 장비 악세사리)를 이용하여, cartridge ①, ②의 실링 필름에 구멍을 뚫은 후, Cartridge ②의 G1~J1 열에는 page 8~9를 참조하여 Storage buffer를 분주하시기 바랍니다.

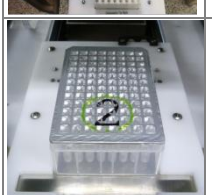
예 1) 1 개의 시료인 경우

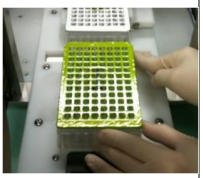
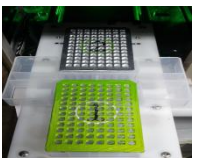
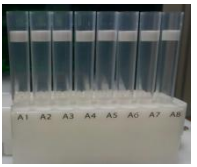


예 2) 8 개의 시료인 경우



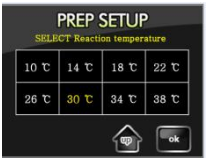
② 이후, 아래의 순서로 *ExiProgen*™ 장비에 장착하시기 바랍니다.

	1. *ExiProgen*™ 장비의 문을 열고 Setup tray를 앞으로 완전히 잡아 당깁니다.
	2. 숫자 ②가 쓰여진 위치에 Cartridge ②를 장착하시기 바랍니다. 주의) Cartridge ②의 L행을 Heating block위의 guard에 끼워 넣어 장착하신 후, Cartridge가 흔들 리지 않는지 확인하시기 바랍니다.

	3. 숫자 ①이 쓰여진 위치에 Cartridge ①을 장착하시기 바랍니다. 주의) Cartridge ① 장착 위치에는 Cartridge 고정을 위한 실리콘 링이 양쪽에 있습니다. 따라서 Cartridge의 왼쪽 면부터 맞춘 후, 오른쪽 면을 눌러서 끼우고, Cartridge가 흔들리지 않는지 확인하시기 바랍니다.
	4. Cartridge ②와 ①을 모두 장착한 후, 사이에 Waste tray를 장착하시기 바랍니다. 주의) Cartridge ② → Cartridge ① → Waste tray 순서를 지켜서 장착하신 후, 흔들리지 않고 제대로 고정이 되었는지, 확인하시기 바랍니다.
	5. 좌측의 그림과 같이 마그네틱 파트가 있는 부분에 Reaction block을 장착하시기 바랍니다. 주의) Reaction block을 장착할 시에는 A열은 장비안쪽, B열은 Tip rack쪽을 향하게 장착되었는지 확인하시기 바랍니다.
	6. Disposable Tip Rack의 **B행**에 sample의 개수에 맞게 Tip을 꽂으시기 바랍니다. 주의) 단, Cartridge의 뚫린 '열'과 같은 '열'에 tip을 꽂으셔야 합니다. 또한, Cartridge 의 뚫지 않은 열에 상응하는 위치에는 tip을 꽂지 마시기 바랍니다.
	7. Setup tray를 밀어 넣고 문을 닫습니다. 단, 이때에 Set up tray는 소리가 날 때까지 밀어 넣으시기 바랍니다.

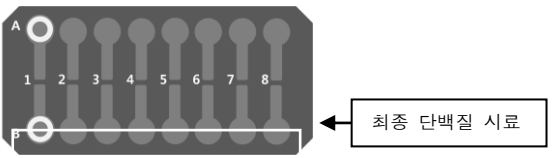
③ 셋팅이 완료된 후, 아래의 순서로 *ExiProgen*™ 장비를 가동하시기 바랍니다.

 ⇩ 	1. *ExiProgen*™ 장비를 전원을 켜고, 'Press to start' 버튼을 눌러주시기 바랍니다. 'start' 버튼을 누르면, 좌측과 같이 *ExiProgen*™ 화면이 뜨고, 스크롤 바가 움직인 후, 다음 화면으로 넘어 갑니다. (이 과정은 장비의 X, Y, Z 축 값을 초기화 하는 과정입니다. 만약 정상적으로 다음화면으로 넘어가지 않는 경우에는 장비의 전원을 끄고, A/S센터로 연락하시기 바랍니다.)
	2. MENU 화면에서 'Start' 버튼을 누르면, 프로토콜을 선택할 수 있는 다음화면으로 넘어 갑니다.
	3. 좌측 화면과 같이 PREP SETUP 화면이 나타나고, 각 키트에 해당하는 프로토콜 번호를 선택할 수 있는 화면이 나타납니다. 여기에서 **'903'**을 눌러 화면에서 **Prep type: Protein** **Sample SRC; Synthesis_Maxi** 가 나타나는지 확인해 주시기 바랍니다. 그 후, 'Enter' 버튼을 눌러주시기 바랍니다.

	4. 프로토콜 선택 후, Elution volume을 선택할 수 있는 화면이 나타나는데, 이는 핵산 추출 시 사용되는 것으로 본 키트 사용시에는 곧바로 'ok'버튼을 눌러 다음 단계로 이동하시기 바랍니다.
	5. Elution volume 선택 화면 후, 단백질 합성 시 온도를 선택할 수 있는 화면이 나타나며, 본 키트 사용시에는 **30℃**를 선택하신 후, 'ok'버튼을 눌러주시기 바랍니다.
	6. 'CHECK LIST'화면이 뜨면, Cartridge 와 Reaction block 등을 각 위치에 맞게 셋팅 되어 있는지 확인하신 후 'ok' 버튼을 눌러 주시기 바랍니다.
 	7. 좌측과 같이 'Running Mode' 화면이 뜨면, 최종적으로 **Prep type: Protein과 Sample SRC; Synthesis_Maxi 가 맞는지 확인하신 후, 'RUN'**버튼을 눌러 주시기 바랍니다. 그 후, 장비 가동이 시작되고 프로토콜 실행이 완료 되면 좌측 하단의 그림처럼 'Work Completion' 화면이 나타납니다. 실험에 사용한 모든 부속품을 제거하신 후, 원하시는 버튼을 눌러주시기 바랍니다. (단, 종료를 원하시어 'ok'버튼을 누르시면, UV lamp가 가동됩니다.)

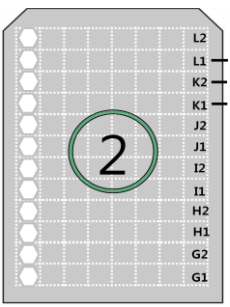
3.4.4. Analysis of sample

ExiProgen™ 장비를 이용한 단백질 합성이 끝난 후, 최종 목적 단백질은 Reaction block의 **B행의 tube**에서 회수하실 수 있으며, 단백질 용액은 약 220~230 uL 정도가 회수됩니다. (단, 최종 단백질 용액에는 Ni-magnetic magnetic bead가 포함되어 있을 수 있으나, 이는 centrifuge를 통해 제거하신 후 사용하시면 됩니다.)



또한 단백질 발현 및 정제 과정 중의 일부 시료는 그림 2와 같이 Cartridge ②의 L1, K1, K2행에서 회수하실 수 있습니다.

그림 2. Cartridge ②의 각 행별 시료



Expression sample
Unbound sample
1st washing sample

*** Expression sample**
; 단백질 발현 시료
*** Unbound sample**
; 발현시료를 Ni-NTA bead에 결합시킨 후의 상등액 (bead에 결합하지 않은 단백질을 포함하는 용액)
*** 1st washing sample**
; 단백질 정제시, 1차 washing 후, 상등액

각 시료는 SDS-PAGE를 통해 원하는 단백질의 합성이 제대로 이루어 졌는지 확인할 수 있습니다.

a. Loading mixture를 아래와 같이 제조하시기 바랍니다.

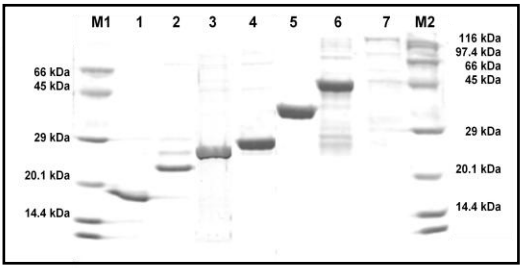
	최종 단백질 시료	Expression/Unbound/ 1st washing samples
sample	5 uL	5 uL
4x loading dye	5 uL	5 uL
멸균증류수	10 uL	10 uL
Total	20 uL	20 uL

b. 95℃에서 5~10min 분간 열처리를 하시기 바랍니다.

c. 10% 또는 12% SDS-PAGE gel(10x8 (cm), 10 well)에 각 시료를 하기와 같은 양을 loading 하신 후, running 하시기 바랍니다.
최종 단백질 시료 ; 10 uL,
Expression/Unbound/1st washing sample ; 5uL

d. Coomassie blue 용액으로 염색 및 탈색하시면 그림 3 과 같이 목적단백질의 밴드를 확인하실 수 있습니다.

그림 3. 단백질 합성 확인



M1; AccuLadder™ Protein Size Marker (Low), M2 ; AccuLadder™ Protein Size Marker (Broad), 1; CalmL3 (17.5kDa) 2; DUSP 3 (22kDa), 3; CAT (24kDa), 4; AcGFP (29kDa), 5; EF-Ts (34kDa), 6; VF (45kDa), 7; BM3 (117kDa)

4. Maintenance

단백질 합성이 끝난 후, 반응 블록 및 기타 장비 액세서리는 다음과 같이 세척 후 보관하시면 됩니다.

A. Reaction Block

단백질 합성이 끝난 후, Reaction Block은 사용한 모든 웰을 멸균 증류수로 세척한 후, 70% 에탄올 용액에 담근 상태로 상온에서 보관하시면 됩니다. 사용 전에 꺼내어, 멸균 증류수로 세척한 후 말린 후 사용하시면 됩니다.

B. Waste tray 및 Disposable tip rack

Waste tray는 tray 안의 용액을 버리고 흐르는 물에 씻은 후 20% ethanol을 뿌려서 닦은 후, 보관하면 됩니다. 또한 Disposable tip rack은 불순물이 묻어 있지 않은 경우에는 그대로 보관하시고, 만약 불순물이 묻은 경우에는 20% Ethanol을 뿌려서 닦은 후, 보관하면 됩니다.

C. Cartridge ①, ②

반응이 끝나고, 남은 Cartridge는 뚜껑을 덮은 상태로, 각각의 온도에 맞게 냉장 또는 냉동 보관 하시기 바랍니다.

5. Troubleshooting guide

단백질 합성에 문제가 있는 경우, 아래의 내용을 참조하여 해결하시기 바랍니다. (단, 아래의 내용은 일반적인 단백질의 합성에 대한 해결방법을 제시해 줄 수 있으나, 모든 단백질의 합성 문제의 해결 방안에 해당되지는 않을 수 있다는 점을 유의하시기 바랍니다.)

1. Positive control 단백질의 합성이 되지 않는 경우

원인	해결방안
핵산분해효소(DNase, RNase)의 오염	실험을 하실 때에는 항상 장갑을 끼고, DNase-, RNase-free한 pipette tips을 이용하시기 바랍니다.
Pipetting error 또는 시약 미첨가	실험을 하기 전 pipettes을 체크하시고, 정확한 양의 DNA 또는 시약을 첨가하시기 바랍니다. 또한 프로토콜에 따라서 모든 시약이 빠짐없이 혼합되었는지 확인하시기 바랍니다.
시약의 보관상태	키트내의 모든 시약 및 구성품은 권장하는 온도에서 보관하시기 바라며, 특히 E. coli extract는 freezing/thawing을 반복하지 않도록 주의하시기 바랍니다.

2. Positive control 단백질은 합성되나, 목적단백질이 합성되지 않는 경우

원인	해결방안
Template DNA의 염기서열	Target gene을 포함하는 코돈의 프레임(ATG (start) ~ TAA, TGA, TAG (Stop))이 올바른지 확인하시기 바랍니다. Template DNA의 ORF (Open Reading Frame)에 mutation이 생기는 경우 translation 중간에 합성이 끝날 수 있으니, 반드시 ORF의 서열을 확인하시기 바랍니다.

발현벡터의 구조	목적단백질을 발현할 수 있는 유전자는 T7 promoter와 terminator가 있는 벡터 (ex) pBIVT, pK7, pIVEX, pET 일부)에 클로닝하여 사용하여야 하며, endogenous lac repressor가 생성될 수 있는 벡터의 경우에는 IPTG를 첨가하여 합성을 하셔야 합니다.
Template DNA의 오염	Template DNA를 준비하는 과정에서 핵산분해효소의 오염에 의해서 DNA가 분해된 경우에는 단백질 합성이 되지 않습니다.

3. 목적 단백질의 합성량이 낮은 경우

원인	해결방안
Template DNA의 순도	최적화된 단백질 합성을 하기 위해서는 template DNA의 순도가 높아야 합니다. $A_{260/280}$값이 1.7~2.0, $A_{260/230}$ 값이 1.5 이상일수록 합성 효율이 높아집니다. 만약 DNA의 순도가 낮은 경우에는 단백질의 합성이 되지 않을 수도 있습니다.
Template DNA의 농도	Template DNA의 농도에 따라서 단백질의 합성량에 차이가 있을 수 있습니다. 단백질 합성량의 최대화하기 위해서는 실험에 앞서 발현 스크리닝을 통해서 최적 DNA 농도를 결정한 후, 단백질 합성을 하시기 바랍니다. (Appendix 2. 참조)
Template DNA의 *E. coli* 코돈 최적화 여부	Template DNA의 염기 서열이 *E. coli* 코돈 최적화가 이루어지지 않은 경우에는 단백질의 합성이 되지 않거나, 합성량이 낮을 수 있습니다.

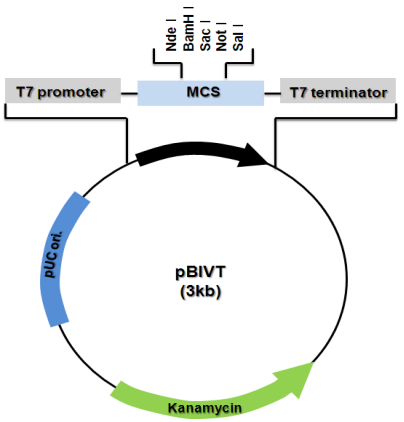
histidine tag의 위치	Histidine tag의 위치에 따라서 목적단백질의 발현을 저해시킬 수 있으며, 단백질의 발현에는 영향을 주지는 않으나, 3차 구조 형성 시 외부로 노출되지 않아서 정제가 되지 않는 경우가 생길 수 있습니다. 이러한 경우에는 tag의 위치를 변경하시기를 권장합니다.

4. 목적 단백질의 활성 또는 solubility가 낮은 경우

원인	해결방안
Posttranslational modification을 필요로 하는 단백질인 경우	*E. coli*를 이용한 무세포 단백질 합성시스템에서는 glycosylation, phosphorylation 등과 같은 posttranslational modification이 필요한 단백질은 합성이 불가능합니다.
단백질 활성에 특정 요소를 필요로 하는 경우	합성된 단백질이 활성을 나타내기 위해서 특정 요소 등을 필요로 하는 경우에는 최종적으로 정제된 단백질 용액에 이러한 요소들을 첨가해준 후 활성을 확인하셔야 합니다.
단백질의 solubility가 낮아서 aggregation이 되는 경우	단백질 합성 온도를 낮춰주거나, 단백질의 folding을 도와주는 chaperone protein을 첨가한 후 합성을 하면, solubility가 향상될 가능성이 있습니다.

6. Appendix 1 : pBIVT vectors

당사의 무세포 단백질 합성 전용 벡터인 pBIVT 벡터 세트는 *ExiProgen*™ 단백질 합성시스템의 모든 키트에 적용 가능합니다. 본 벡터 세트는 N-terminal 또는 C-terminal 에 6x histidine tagging 이 가능하며, 구조는 아래와 같습니다.



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

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

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

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

7. Appendix 2
: Determination of template DNA concentration

ExiProgen™ 단백질 합성시스템을 이용하여 단백질을 합성하실 때, template DNA의 양에 따라서 단백질 합성량에 차이가 있을 수 있습니다.

Template DNA는 하기와 같이 농도에 따른 발현 스크리닝 먼저 선행하신 후, *ExiProgen*™을 이용한 단백질 합성을 하시면 최적의 단백질 합성 결과를 얻으실 수 있습니다.

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

1. Template DNA를 각 농도별로 준비합니다.
   Ex) 25, 50, 100, 200, 300, 400 (ng/uL)
2. Kit의 매뉴얼에 따라서 단백질 발현 실험 진행을 진행합니다.
   ① Reaction Mixture 준비
   - Reaction volume ; 45uL
   - Sample DNA
   ; 1번에서 준비한 DNA를 각 2uL씩 첨가
   ; Final DNA 농도
   Ex) 50, 100, 200, 400, 600, 800ng
   ② PCR machine을 이용하여 30℃ 에서 3시간 동안 반응을 시킵니다.
   ③ SDS-PAGE gel을 통해서 최적 발현 농도를 확인합니다.

ExiProgen™ EC-Maxi Protein Synthesis Kit

3. 이전 실험을 통해 확인한 최적 DNA 농도의 **10배수** 에 해당하는 DNA 농도를 확인합니다.
4. 위에서 결정한 농도의 DNA를 *ExiProgen*™ EC-Maxi Protein Synthesis Kit 에 첨가하여 단백질을 합성합니다.

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9. Related products

Cat. No.	Product	Size
K-7250	*AccuRapid*™ Cell-Free Protein Expression Kit	45 uL x 24 reactions
K-7260	*AccuRapid*™ Midi Protein Expression Kit	1mL x 5 reactions
K-7270	*AccuRapid*™ Maxi Protein Expression Kit	10mL x 1 reaction
K-7300	*ExiProgen*™ EC Protein Synthesis Kit	16 reactions
K-7301		32 reactions
K-7302		96 reactions
K-7320	*ExiProgen*™ EC-Tagfree Protein Synthesis Kit	8 reactions
K-7330	*ExiProgen*™ EC-Disulfide Protein Synthesis Kit	8 reactions
K-7350	pBIVT vector set	Each 5ug
K-7400	*ExiProgen*™ ProXpress PCR Template Kit	16 reactions
K-7401		32 reactions
D-2010	*AccuLadder*™ Protein Size Marker (Broad)	500 uL
D-2020	*AccuLadder*™ Protein Size Marker (Low)	500 uL
S-2041	Gene Synthesis Service	-
S-2500	Protein Synthesis Service	-
A-5041	*ExiProgen*™	-

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