Ver. 1.0

User's Guide

Global Genomics Partner

ExiProgen™ EC1 Protein Synthesis Kit

BIONEER

Innovation • Value • Discovery

Safety Warnings and Precautions

ExiProgen™ EC1 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.

Trademarks

ExiProgen™ is trademark of Bioneer Corporation.

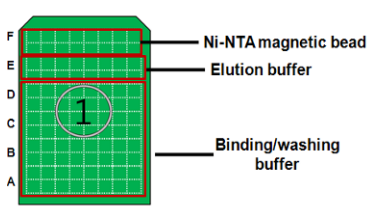
CONTENTS

1. KIT CONTENTS AND STORAGE

1.1. Kit Contents

***ExiProgen*™ EC1 Protein Synthesis Kit – 1**		K-7300 (16 reaction)	K-7301 (32 reaction)	K-7302 (96 reaction)
Cartridge ①	Ni-NTA magnetic bead (500 ul/well)	16well x 1ea	16well x 2ea	16well x 6ea
	Binding/Washing buffer (1.2 ml/well)	64well x 1ea	16well x 2ea	16well x 6ea
	Elution buffer (250 ul/well)	16well x 1ea	16well x 2ea	16well x 6ea
Disposable filter tip	33tips/pack	1/2 pack	1 pack	3packs
***ExiProgen*™ EC1 Protein Synthesis Kit - 2**		K-7300 (16 reaction)	K-7301 (32 reaction)	K-7302 (96 reaction)
Cartridge ②	Master mix (350 ul/well)	16well x 1ea	16well x 2ea	16well x 6ea
	DEPC DW (250 ul/well)	16well x 1ea	16well x 2ea	16well x 6ea
Cell extract	8-strip tube (200 ul/tube)	2ea	4ea	12ea
Positive control DNA	10 ug (1.5 ml tube)	1ea	1ea	1ea
Elution tube/cap	8-strip	2ea	4ea	12ea

< Cartridge ① >



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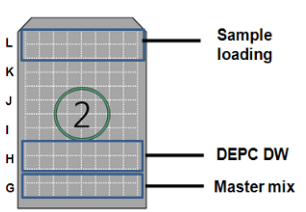


1.2. Storage

ExiProgen™ EC1 Protein Synthesis Kit consists of a Purification Kit (Kit No. 1) and an Expression Kit (Kit No. 2).

ExiProgen™ EC1 Protein Synthesis Kit No.1 is for protein purification, and contains Disposable filter tips and Cartridge ① (Ni-NTA magnetic beads, Binding/Washing buffers and Elution buffers). Cartridge ① must be stored at 4℃.

ExiProgen™ EC1 Protein Synthesis Kit No. 2 is for protein expression, with Cell Extracts, Positive Control DNA, Elution tubes/caps and Cartridge ② (Master Mix and DEPC water). Cartridge ② and Cell extracts must be stored below -20℃.
(We recommend storing the Cell Extract below -70℃)

The cartridges within the kit are sealed with sealing film to prevent cross-contamination, evaporation and leaking. All plastics and buffers are DNase- and RNase-free. Please take caution not to contaminate the components with nucleases or proteases.

2. INTRODUCTION

2.1. Overview

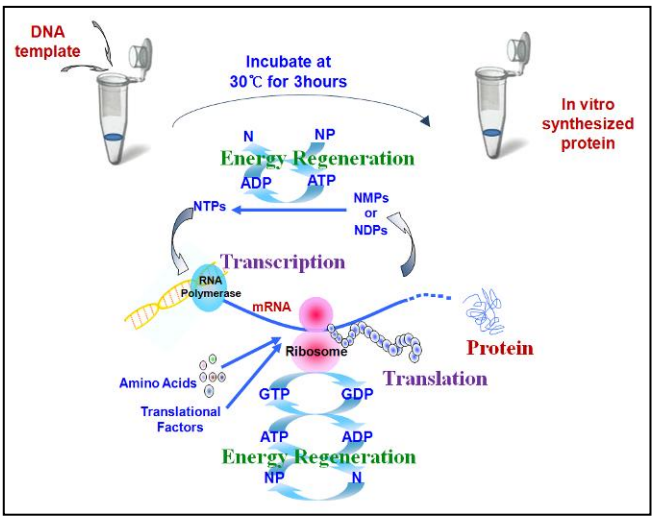
As life technology has developed over the years, research into genes and their protein products have continued. While genetic technologies based on DNA modification was mainstream during the past, research into protein-level mechanism studies is becoming more and more popular.

In the past, *in vivo* expression using cells was the method of choice for protein expression. This method involves the transfection of recombinant DNA into the host cell, cell culture and subsequent purification. Protein expression by cell culturing is a time-consuming process and is in many cases not economical.

To overcome this shortcoming, *in vitro* transcription/translation methods have been developed, which use cell extracts and ingredients essential for protein synthesis mixed in a tube. When recombinant DNA containing the protein codon of interest is added and the mixture is maintained at a certain temperature, the desired protein is synthesized in an efficient manner (Figure 1). Because this method does not require a separate cell line selection step, it is able to yield various types of protein in a short period of time. *In vitro* protein expression can also express proteins that are difficult to over-express due to cellular toxicity effects.

The continuous development of *in vitro* (cell-free) protein expression is revolutionizing the once time-consuming protein modification research field, and will have far-reaching implications not only in academic research but also industrial enzyme development as well.

Figure 1. *In vitro* expression of recombinant protein



3. *ExiProgen*™ EC1 Protein Synthesis Kit

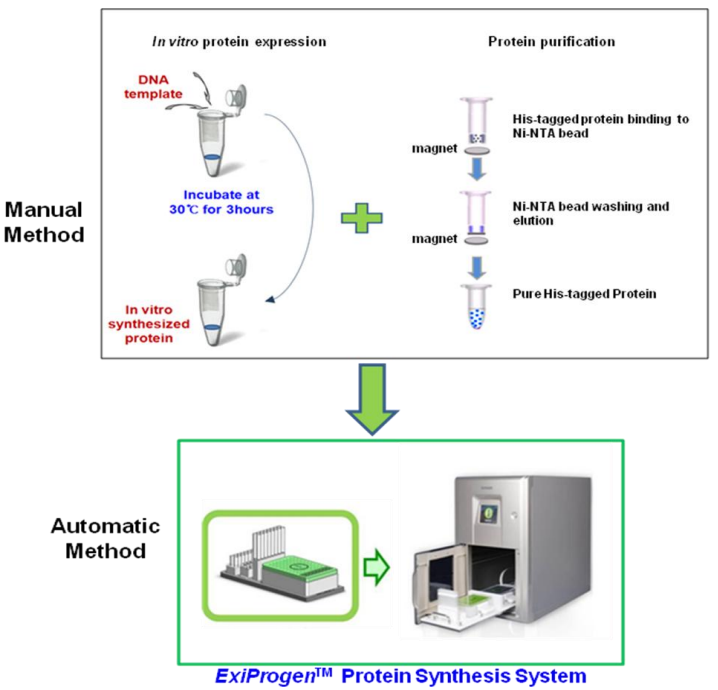
3.1. Principle of *ExiProgen*™ Protein Synthesis System

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 2.

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 2. Principle of *ExiProgen*™ Protein Synthesis System



ExiProgen™ EC1 Protein Synthesis Kit contains a Cell Extract (8-well strip tube) which has T7 RNA Polymerase and Ribosomes, Cartridge ② which contains a Master Mix (components necessary for RNA and protein synthesis) and DEPC DW, and Cartridge ① which contains Ni-NTA magnetic beads and buffers for His-tag purification. (The Ni-NTA magnetic beads are suspended in 20% ethanol at 10% (w/v))

3.2. Information of Template DNA

The template DNA for expression by *ExiProgen*™ EC1 Protein Synthesis Kit must have a " T7 promoter - Ribosome binding site (RBS) – Target gene - T7 terminator " structure, and the target gene must contain a start/stop codon, a Histidine tag (N- or C-terminus). The structure is shown in Figure 3.

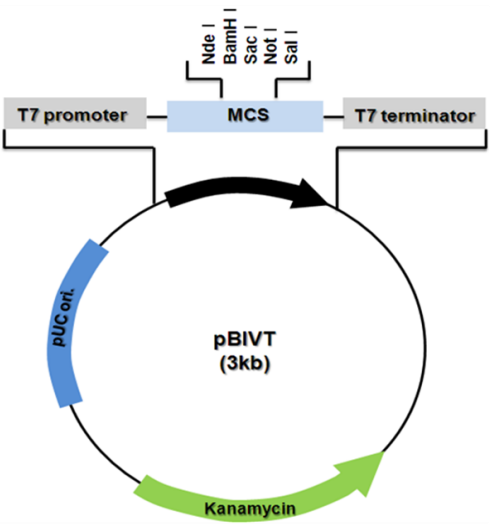
Figure 3. Structure of template DNA

Note 1) We recommend that the *in vitro* translation vector (pBIVT vector set) by Bioneer be used (refer to Figure 4) for the best results.

Note 2) The DNA template for protein synthesis can be optimized for *E. coli* expression using our Gene Synthesis Service, and can be cloned in our *in vitro* translation vector as an option. Please refer to our homepage (www.bioneer.com) for details.

Note 3) *E.coli in vitro* translation vectors such as pK7, pQE, pIVEX etc. can also be used.

Figure 4. Vector map for *ExiProgen*™ EC1 Protein Synthesis Kit



1) MCS site

pBIVT 1 : Nde I - 6xHis - BamH I - Sac I - Not I - Stop codon - Sal I
pBIVT 2 : Nde I - BamH I - Sac I - Not I - 6xHis - Stop codon - Sal I

2) Sequences

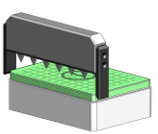
pBIVT 1 : CATATGCACCACCACCACCACCACGGATCCGAGCTCAAGCTTGCGGCCGCATAGGTCGAC
pBIVT 2 : CATATGGGATCCGAGCTCAAGCTTGCGGCCGCACACCACCACCACCACCACTAGGTCGAC

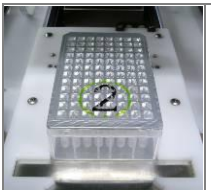
3.3. Protocol

1. Prepare 6~10ug of the Positive control DNA and sample DNA.
   (Ex. 30~50ul of 200ng/ul DNA, or 6~10ul of 1ug/ul DNA)

2. Before use, thaw Cartridge ② at room temperature for 20 minutes.

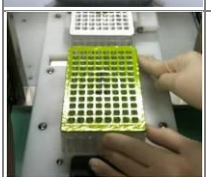
3. Thaw the Cell Extract on ice. Lightly tap and spin-down before use.
   Do not vortex

	1. Use the hole-punch to punch the appropriate number of holes of the sealing films of Cartridges ① and ②. * Briefly spin-down both cartridges to ensure the magnetic beads and buffers remain at the bottom of the wells.
	2. Thaw the sample DNA and briefly spin-down before use.
	3. Pipette the appropriate amount of DNA in row L of Cartridge ②. (Use 10ul of Control DNA)



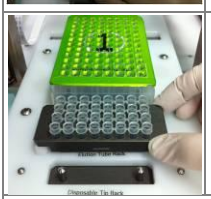
4. Open the door of *ExiProgen*™, pull out the Base Plate and load Cartridge ② in the prescribed position.

∗ Ensure that the sealing film of Cartridge ② is correctly punched.



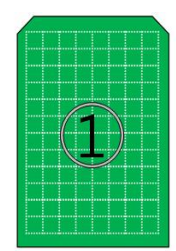
5. Load Cartridge ① in the prescribed position on the Base Plate.

∗ Ensure that the sealing film of Cartridge ① is correctly punched.



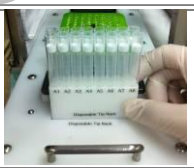
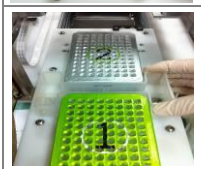
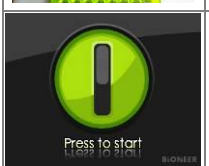
6. Load the Elution Tube Rack with the Cell Extract and Elution Tubes on the prescribed position on the Base Plate.

∗ Confirm that the Cell Extract and Elution Tubes are in the correct positions (Below).



※ Caution!!!

ExiProgen™ uses a Cooling Block to maintain the Cell Extract and eluted proteins at low temperatures.
The Cell Extract is positioned below Cartridge ①.
Refer to the diagram on the left to place the Cell Extract and Elution tubes in their correct positions.

	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.
	8. Place the Waste tray between Cartridge ① and Cartridge ②. Push the Base Plate inside the instrument and close the door.
	9. Turn on the instrument power and tap the icon on the screen to initialize the instrument.
	10. Tap the 'Start' icon on the MENU to proceed with PREP SETUP.
	11. Enter the Protocol number No. 902 and tap 'Enter' to proceed.

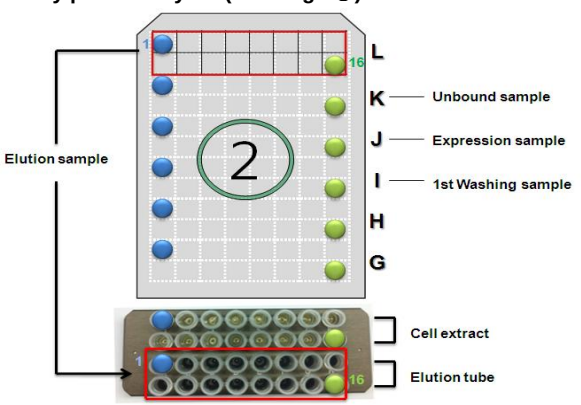
	12. Set the Elution volume at '250 ul' and tap 'OK' to proceed.
	13. Verify that all the items on the 'CHECK LIST' have been completed before tapping 'OK' to proceed with the experiment.
	14. Verify the following information in the 'Running Mode' screen: **Prep type : Protein, Sample SRC : Synthesis** Press ''RUN' to initiate synthesis.
	15. After the run is complete, remove all components from within the instrument including the Elution tubes before tapping 'OK' to finish the program. * If you wish to perform additional work, tap the according option number instead.

3.4. Analyzing Samples

After expression and purification of proteins using *ExiProgen*™, you may use SDS-PAGE to determine the success of synthesis, and Bradford or Lowry methods can be used to quantify the amount of purified protein.

The purified protein can be harvested from the Elution Tubes, and other by-products of protein synthesis and purification can be obtained from the corresponding wells of Cartridge ② (Figure 5).

Figure 5. By-product layout (Cartridge ②)



- Expression sample: Total protein product of *in vitro* translation
- Unbound sample: Sample fraction that was not bound to the Ni-NTA beads during purification
- 1st Washing sample: Sample fraction that has been washed out during the 1st washing step.
- Six holes are punched with one punch of the 6 hole puncher.
- The final eluted sample is laid out in the Elution Tubes according to the sample input order in section L.

Note) Preparing the sample for SDS-PAGE

1. Verifying samples from sections I, J and K of Figure 5.
   ; [5 uL '4x Loading dye' + 5 uL 'sample' + 10 uL 'sterile DW'].
2. Elution sample
   ; [5 uL '4x Loading dye' + 15 uL 'Elution sample'].
3. Denature all samples at 95℃ for 5 minutes and load 5 uL/well for sample type 1, and 10 uL/well for sample type 2.

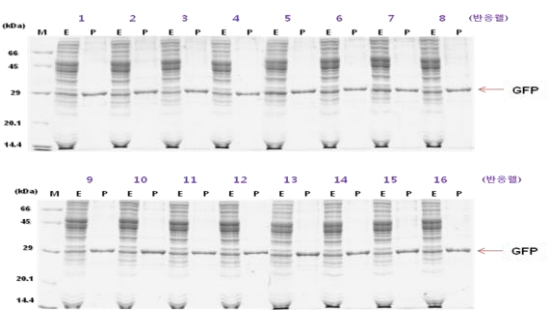
3.4.1. Experimental Data

This product can synthesize and purify over 100 ug of up to 16 different types of proteins simultaneously that are over 90% pure within 6 hours.

The results of protein synthesis and purification using this product are shown in Figures 6 and 7.

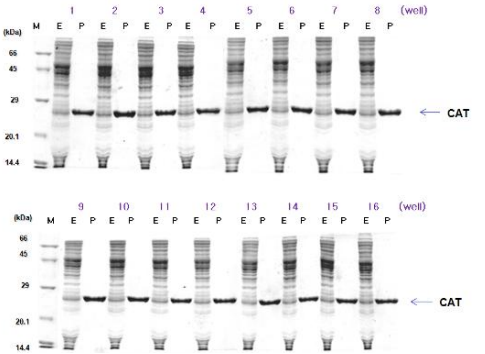
1. <u>The same template can be synthesized in all 16 wells without well-to-well variation.</u>

Figure 6-1. Expression and purification of GFP (Green Fluoresce Protein)



M : *AccuLadder*™ Protein Size Marker (Low)
E : Expression sample, P : Purification sample

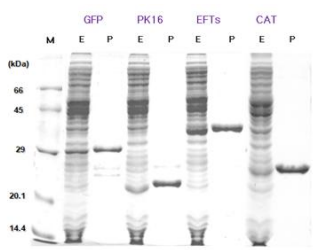
Figure 6-2. Expression and purification of CAT (Chloramphenicol Acetyl Transferase)



M : *AccuLadder*™ Protein Size Marker (Low)
E : Expression sample, P : Purification sample

2. <u>Several different proteins can be synthesized for ~100 ug of over 90% pure protein.</u>

Figure 7. Expression and purification of various His-tagged proteins



M : *AccuLadder*™ Protein Size Marker (Low)
E : Expression sample, P : Purification sample

4. TROUBLESHOOTING

1. Synthesis of the desired protein

1) Verifying the DNA template

① Please verify the concentration and purity of the DNA template. (Please verify on an agarose gel)

Note) When using the *ExiProgen*™ EC1 Protein Synthesis Kit, the DNA template purity must be above 1.8.

② Verify the sequence of the plasmid DNA.

- Verify that the start and stop codons of the target gene are correct.

2) Verifying the location of the Histidine tag within the target gene

- The location (N- or C-terminus) of the Histidine tag may interfere with expression, structure or purification.

2. Purity and amount of desired protein

1) Nuclease contamination

- If nuclease contamination occurs during the course of the experiment, the DNA template may degrade and decrease the yield of the desired protein.

2) Verifying DNA template concentration

- To maximize protein yield, an optimal concentration of DNA is necessary (6~10 ug).

Ex) CAT : 6ug, PK16 : 6ug, GFP : 10ug, EF-Ts : 10ug

3) Purity and Yield

- Purity and yield may vary depending on protein type.

5. Ordering Information

Product	Size	Cat. No.
ExiProgen™ EC1 Protein Synthesis Kit	16 reaction	K-7300
	32 reaction	K-7301
	96 reaction	K-7302
pBIVT vector set-1	Each 5ug	K-7350
Gene Synthesis Service	-	S-2041
ExiProgen™	-	A-5041