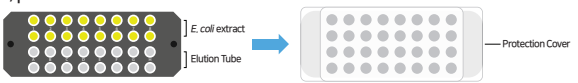
ExiProgen™ EC Protein Synthesis Kit (Cat.No. K-7300, K-7301, K-7302)

Step I Preparation of Template DNA

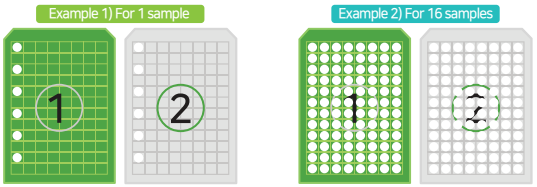
1) Prepare the 'Template DNA (1-10 µg)' for protein expression.
(Note I) The 'Template DNA' must contain the following: T7 Promoter, Ribosome binding site (RBS), Target gene (with His-tag), and T7 terminator.
(Note II) Use 1 µg/kb of plasmid DNA or 0.5 µg/kb of PCR product (up to 1.5 kb of PCR product). (Purity: $A_{260/280}$ >1.7, $A_{260/230}$ >1.5)

Step II Preparation of Experiment

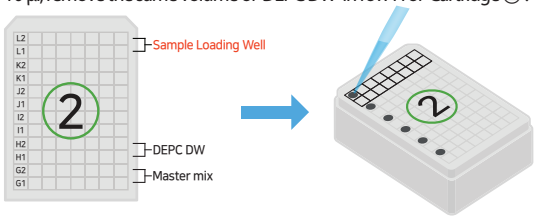
1) Take out 'Cartridge ②' from **Box** ②. Thaw them at room temperature.
2) Take out '*E. coli* extract' from **Box** ②. Thaw it on ice.
Take out 'Cartridge ①', 'Disposable Filter Tip', 'Elution Tube', and 'Protection Cover' from **Box** ① and **Box** ②.
(Note) Make sure that all the solutions are completely thawed. It takes about 2 hours.
3) Load '*E. coli* extract' and 'Elution Tube' on the 'Elution Tube Rack (*ExiProgen*™ accessory)'.
(Rows in number: *E. coli* extract, Rows in alphabet: Elution Tube)
Then, place the 'Protection Cover' on the 'Elution Tube Rack'.



4) Punch holes in the sealing films of 'Cartridge ①, ②'.
(Note) The number of columns punched should match the number of samples used in this test.



5) Add the 'Template DNA' to 'Sample Loading Well' of 'Cartridge ②'.
(Note) Use 10 µl of Positive Control DNA. If the volume of 'Template DNA' is more than 10 µl, remove the same volume of 'DEPC DW' in row H of 'Cartridge ②'.



Step III Protein Synthesis with *ExiProgen*™

1) Turn on the *ExiProgen*™ and tap 'Press to Start' button.
Wait until the [MENU] screen appears.
2) Open the door of *ExiProgen*™ and pull out the baseplate.

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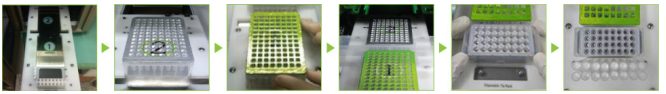
3) Load 'Cartridge ①, ②', 'Waste Tray', and 'Elution Tube Rack'.
Follow in order: 'Cartridge ②' → 'Cartridge ①' → 'Waste Tray' → 'Elution Tube Rack'.
(Note I) Make sure that the first and second lines of 'Cartridge ②' are firmly fixed on the heating block.
(Note II) There are 'silicon rings' embedded on both sides of the 'Cartridge ①' installation position. Place the left side first, and then the right side.

4) Place the 'Disposable Filter Tips' on **row A or B** of 'Disposable Tip Rack'.
(Note) Tips should be placed in the same columns with the punched holes of the Cartridges.



5) Push the baseplate in until you hear the click sound, then close the door.

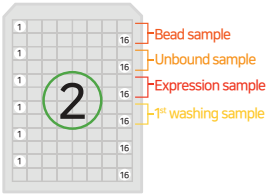
6) Tap the following: [MENU] 'Start' → [PREP SETUP] **'902 (Protein Synthesis)'**, 'Enter' → [PREP SETUP, Elution Volume] 'OK' → [PREP SETUP, Reaction Temperature] '30°C', 'OK' → [CHECK LIST] 'OK' → [Running Mode] 'RUN'.

7) The [Work Completion] screen will appear once the protocol is completed.
It takes approximately **6 hours**.

8) Purified protein samples in the elution buffer can be collected from the elution tubes in the rack, located at the **rows labeled with alphabets**.

Step IV Analysis of Sample

A. Position of each sample (in 'Cartridge ②')



Expression sample
: Samples after expression, but without purification
Unbound sample
: Samples not bound to Ni-NTA magnetic bead
1st washing sample
: Samples after 1st washing step in purification process
Bead sample
: Used bead samples for purification

B. Sampling for SDS-PAGE analysis

1) Prepare the loading mixture as shown in the table. Incubate the samples at 95°C for 5-10 min.

	Expression/Unbound/1st washing sample	Purified protein/Bead sample
Sample	5 µl	15 µl
4X Loading dye	5 µl	5 µl
Sterile distilled water	10 µl	-
Total volume	20 µl	20 µl

(Note) Add 200 µl of sterile distilled water to the well containing the beads for suspension. Proceed with the sampling afterwards.

2) Load each sample to the wells of SDS-PAGE gel [10 x 8 (cm), 0.75 mm thick, 10-well].
(Note) Expression, Unbound and 1st washing samples: 5 µl/well,
Purified proteins and Bead samples: 10 µl/well.

※ For more information, visit our **website (www.bioneer.com)**.