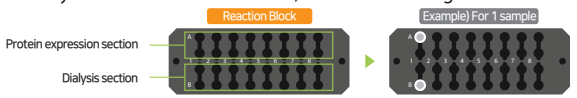
ExiProgen™ EC-Tagfree Protein Synthesis Kit (Cat. No. K-7320)

Step I Preparation of Template DNA

1) Prepare the 'Template DNA' for protein expression.
   (Note I) The 'Template DNA' must contain the following: T7 Promoter, Ribosome binding site (RBS), His tag - TEV cleavage site - Target gene, and T7 terminator.
   (Note II) Use 0.6 μg/kb of plasmid DNA (Purity: $A_{260/280}$ >1.7, $A_{260/230}$ >1.5).

Step II Preparation of Experiment

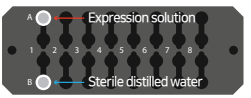
1) Take out the 'Cartridge ②' from **Box ②**. Thaw them at room temperature.
2) Take out '*E. coli* extract', 'Master Mix', 'DEPC DW', 'Storage Buffer' and 'TEV protease' from **Box ②** and thaw them on ice.
   Take out 'Cartridge ①', 'Disposable Filter Tip', and 'Protection Cover' from **Box ①**.
   (Note) Make sure that all solutions are completely thawed. It takes about 2 hours.
3) Prepare the 'Reaction Block (*ExiProgen*™ accessory)'.
4) Take out twice as many 'Dialysis Tube' as the number of samples from **Box ①**.
5) Remove the solution in the 'Dialysis Tube' and rinse inside and out using squeeze bottle with sterile water.
   (Note) The solution in the 'Dialysis Tube' is 20%(v/v) ethanol.
6) Install the 'Dialysis Tube' on the 'Reaction Block', as shown in the diagram.



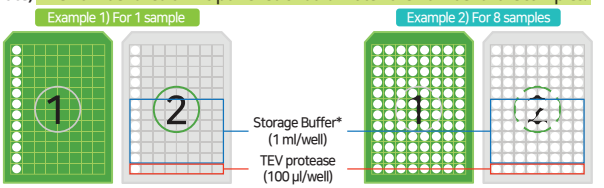
7) Remove the water in the 'Dialysis Tube' and prepare expression solution as shown in the table. The expression solution should be loaded at 'Dialysis Tube' in **row A** (Protein expression section). Fill 500 μl of sterile distilled water in the 'Dialysis Tube' on **row B** (Dialysis section).

*** Composition of Expression Solution**

	Sample	Positive control DNA (Optional)
Template DNA	X μl	6 μl
E. coli extract	120 μl	120 μl
Master Mix	210 μl	210 μl
DEPC DW	(120-X) μl	114 μl
Total volume	450 μl	450 μl



8) Place the 'Protection Cover' on the 'Reaction Block'.
9) Punch holes in the sealing films of 'Cartridge ①, ②'.
10) Add 1 ml of 'Storage Buffer' to G2-J1 rows and 100 μl of 'TEV protease' to G1 row of 'Cartridge ②'.
    (Note) The number of columns punched should match the number of the samples.



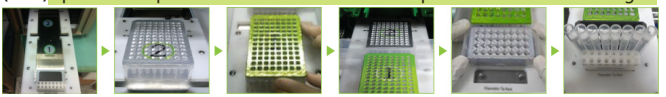
*** Composition of Storage Buffer**
- 50 mM Tris-HCl, 100 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 0.05%(v/v) NaN_3, 50%(v/v) glycerol, pH 7.6

Step III Protein Synthesis with *ExiProgen*™

1) Turn on the *ExiProgen*™ and tap 'Press to Start' button.
   Wait until the [MENU] screen appears.

ExiProgen™ EC-Tagfree Protein Synthesis Kit (Cat. No. K-7320)

2) Open the door of *ExiProgen*™ and pull out the baseplate.
3) Load 'Cartridge ①, ②', 'Waste Tray', and 'Reaction Block'.
Follow in order: 'Cartridge ②' → 'Cartridge ①' → 'Waste Tray' → 'Reaction Block'.
(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 B** of 'Disposable Tip Rack'.
(Note) Tips should be placed at the same columns with the punched holes of the Cartridges.

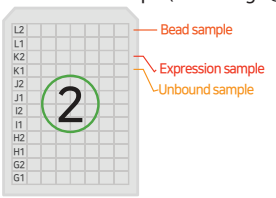


5) Push the baseplate in until you hear the click sound, then close the door.
6) Tap the following: [MENU] 'Start' → [PREP SETUP] '**904 (Synthesis_TF)**,' 'Enter' → [PREP SETUP, Elution Volume] 'OK' → [PREP SETUP, Reaction Temperature] '30°C', 'OK' → [CHECK LIST] 'OK' → [Running Mode] 'RUN'.

7) The [Work Completion] screen will appear once the protocol is completed.
It takes approximately **26 hours**.
8) **Purified protein samples** in the 'Storage Buffer' can be collected from the 'Dialysis Tube', located at the **row B** of the 'Reaction Block'.

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
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 sample	Purified protein sample	Bead sample
Sample	5 µl	5 µl	15 µl
4X Loading dye	5 µl	5 µl	5 µl
Sterile distilled water	10 µl	10 µl	-
Total volume	20 µl	20 µl	20 µl

(Note) Add 360 - 400 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 sample: 5 µl/well,
Purified proteins and Bead samples: 10 µl/well

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