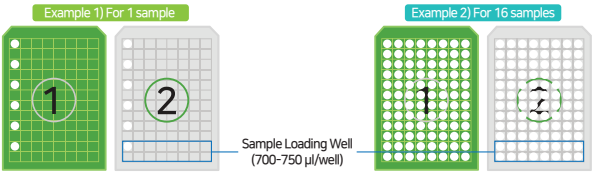
ExiProgen™ His-tagged Protein Purification Kit (Cat.No. K-7220, K-7221)

Step I Sample Preparation (e.g. *E. coli* cell lysate)

1) Harvest the cells, including the target proteins by centrifuging at 3,000 rpm for 10 min.
2) Resuspend the cells with lysis buffer (not provided).
   (Note I) It is recommended to use 10 ml of lysis buffer per 0.1 g of wet cells.
   (Note II) Example of lysis buffer: 20 mM Tris-HCl, 1 mM 2-Mercaptoethanol, pH 7.6
3) Lyse the cells on ice with a sonicator equipped with a microtip.
4) Centrifuge the cell lysate at 13,000 rpm for 1 min to separate supernatant and pellet.
5) Check the presence of target proteins in supernatant with SDS-PAGE.

Step II Preparation of Experiment

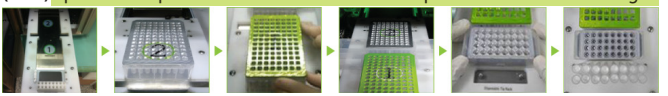
1) Take out 'Cartridge ①, ②', 'Disposable Filter Tip', 'Elution Tube', and 'Protection Cover' from the kit box.
2) Punch holes in the sealing films of 'Cartridge ①, ②'. Add 700-750 µl of samples (e.g. *E. coli* cell lysate) to 'Sample Loading Well' of 'Cartridge ②'.
   (Note) The number of columns punched should match with of the samples.



3) Load 'Elution Tube' on the **'Elution Tube Rack** (*ExiProgen*™ accessory)'.
   (Rows in alphabet: Elution Tube)
   Place the 'Protection cover' on the 'Elution Tube Rack'.

Step III Protein Purification 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.
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.

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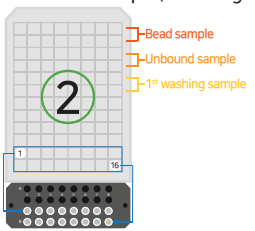
6) Tap the following: 'Start' → [PREP SETUP] **'901 (Protein_Purification)'**, 'Enter' → [PREP SETUP, Elution Volume] 'OK' → [PREP SETUP, Reaction Temperature] 'OK' → [CHECK LIST] 'OK' → [Running Mode] 'RUN'.

7) The [Work Completion] screen will appear once the protocol is completed. It takes approximately **2 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')



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℃ for 5-10 min.

	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) Unbound and 1st washing sample: 5 µl/well,
Purified proteins and Bead samples: 10 µl/well

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