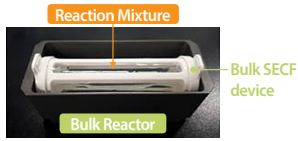
ExiProgen™ EC-Bulk Protein Synthesis Kit (Cat. No. K-7340)

Step I Protein Expression

A. Preparation of Experiment

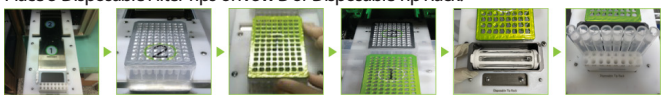
1) Prepare the 'Template DNA' for protein expression.
   (Note) The 'Template DNA' must contain the following : T7 Promoter-Ribosome binding site (RBS)-Target gene (with His-tag)-T7 terminator.
   Use 5-20 µg of plasmid DNA per killobase or 30-50 µg of PCR product.
   (Purity: $A_{260/280}$ >1.7, $A_{260/230}$ >1.5).
2) Take out the 'Expression Cartridge ①, ②' from **Box** ② and thaw them at room temperature.
3) Take out '*E. coli* extract', 'Master Mix', and 'DEPC DW' from a **Box** ② and thaw them on ice.
   (Note) Please make sure that all solutions are completely thawed. It takes about 3-4 hours.
4) Punch all holes in the sealing films of 'Expression Cartridge ①, ②'.
5) Install 'Bulk SECF device' from a **Box** ① on a 'Bulk Reactor (Cat. No. KA-7340-1)' and **fill the 'Bulk SECF device' with the 'Reaction Mixture' as below.**
   (Note) 'Bulk SECF device' is filled with solution containing 0.05% NaN_3.
   Remove the solution in the device before use.

Reaction Mixture	
Template DNA	X ml
E. coli extract	5.34 ml
Master Mix	9.34 ml
DEPC DW	(5.32-X) ml
Total volume	20 ml



B. Protein Expression 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 'Bulk Reactor'.
   Follow in order: 'Cartridge ②' → 'Cartridge ①' → 'Waste Tray' → 'Bulk Reactor'
   , Place 8 'Disposable Filter Tips' on **row B** of 'Disposable Tip 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) Push the baseplate in until you hear the click sound, then close the door.
5) Press [MENU] 'Start' → [PREP SETUP], **'906 (Bulk_Expression)'**, 'Enter' → [PREP SETUP, Elution Volume] 'OK' → [PREP SETUP, Reaction Temperature] '30°C', 'OK' → [CHECK LIST] 'OK'-> [Running Mode] 'RUN'.

6) The [Work Completion] screen will appear once the protocol is completed.
   It takes approximately **25 hours**.
7) Expressed protein samples will be collected in the 'Bulk SECF device'.

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Step II Protein Purification

A. Preparation of Experiment

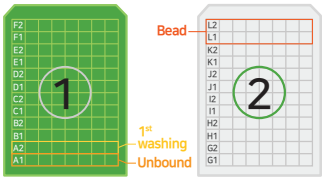
1) Take out 'Purification Cartridge ①, ②' from a **Box** ① and punch all holes in the sealing films.
2) Load the **1 ml/well (Total 16 ml)** expressed protein samples (Final sample of Step I) on the '**Sample Loading Well (16 wells)**' of 'Purification Cartridge ①'.
3) Install the new 'Bulk SECF device' from a **Box** ① on a 'Bulk Reactor (Cat. No. KA-7340-1)' and **fill 20 ml of sterile distilled water in the device**.
   (Note) 'Bulk SECF device' is filled with solution containing 0.05% NaN_3.
   Remove the solution in the device before use.

B. Protein Purification with *ExiProgen*™

1-4) 1-4 steps are the same as 'Protein Expression' protocol. Refer to front page.
5) Press [MENU] 'Start' → [PREP SETUP], '**907 (Bulk_Purification)**', 'Enter' → [PREP SETUP, Elution Volume] 'OK' → [PREP SETUP, Reaction Temperature] 'OK' → [CHECK LIST] 'OK' → [Running Mode] 'RUN'.
6) The [Work Completion] screen will appear once the protocol is completed.
   It takes approximately **15 hours**.
   (Note) The '907' protocol includes [Ni-NTA affinity purification] and [Dialysis to storage buffer] processes. If you want to separate two processes, use [908 (Bulk_Ni-NTA purify)] and [909 (Bulk_Storage)] protocol.
7) Purified protein samples in storage buffer will be collected in the 'Bulk SECF device'.
   (Note I) Purified protein samples may contain trace amounts of magnetic beads. The beads do not influence to proteins and can be easily removed through spin-down.
   (Note II) Composition of Storage Buffer: 50 mM Tris-HCl,100 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 0.05% NaN_3, 50% Glycerol, pH 7.6.

Step III Analysis of Sample

A. Position of each sample (in 'Purification Cartridge ①, 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 loading mixture as shown in the table.

	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 360-400 µl/well of sterile distilled water to the well containing the beads for suspension. Proceed with the sampling afterwards.

2) Incubate the samples at 95°C for 5-10 min.
3) Load 5 µl of each sample to a well of SDS-PAGE gel [10 x 8 (cm), 0.75 mm thick, 10-well].

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