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 should have the structure of "T7 Promoter-RBS-Target gene (with His-tag)-T7 terminator".*
   Use 5~20 μg of plasmid DNA(per killobase) or 30~50μg of PCR product.
   (Purity-$A_{260/280}$; >1.7, $A_{260/230}$; >1.5).

2) Take out 'Expression Cartridge ①, ②' from a **Box ②** and thaw them at the room temperature.
   Note) *Please make sure that all solutions are completely thawed. It takes about 3~4 hours.*

3) Punch all holes in the sealing films of 'Expression Cartridge ① and ②'.

4) Take out '*E. coli* extract', 'Master mix' and 'DEPC DW' from a **Box ②** and thaw them on ice.

5) Install 'Bulk SECF device from a **Box ①**' on a 'Bulk Reactor (Cat. No. KA-7340-1)' and **Fill the Reaction mixture into the device.**
   Note) *'Bulk SECF device', is filled with distilled water containing 0.05% NaN_3. Remove the water in the device before use.*

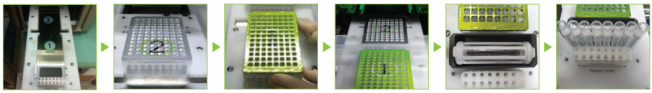
Reaction mixture	
Template DNA	X mL
E.coli extract	5.34 mL
Master mix	9.34 mL
DEPC DW	(5.32-X) mL
Total	20 mL

B. Protein Expression with *ExiProgen*™

1) Turn on the *ExiProgen*™ and press the 'Press to start' button.
   A loading bar will appear on the screen. Afterwards, the [MENU] screen will appear.

2) Open the door of the *ExiProgen*™ and pull out its base plate.

3) Install 'Cartridge ①, ②', 'Waste tray', and 'Bulk Reactor'.
   Follow the exact order: 'Cartridge ②' → 'Cartridge ①' → 'Waste tray' → 'Bulk Reactor'.
   And place **8 tips** of 'Disposable filter tips' on **B row** 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 in both sides of the installation position of 'Cartridge ①'. Install the left side first and then the right side.*

4) Push the Base plate completely, until you hear 'click' sound or feel clicking, and close the door.

5) Press [MENU] 'Start' → [PREP SETUP], **'906 (Bulk_Expression)'**, 'Enter' → [PREP SETUP, Elution Volume] 'OK' → [PREP SETUP, Reaction Temperature] '30 ℃, 'OK' → [CHECK LIST] 'OK'-> [Running Mode] 'Run'.

6) About **25 hours** later, [Work Completion] screen will appear when the protocol is Completed.

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 of them.

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 '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 distilled water containing 0.05% NaN_3. Remove the water 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.

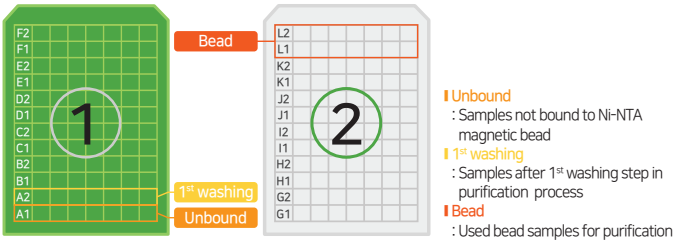
5) Press [MENU] 'Start' → [PREP SETUP], **'907 (Bulk_Purification)'**, 'Enter' → [PREP SETUP, Elution Volume] 'OK' → [PREP SETUP, Reaction Temperature] 'OK' → [CHECK LIST] 'OK' → [Running Mode] 'Run'.

6) About **15 hours** later, [Work Completion] screen will appear when the protocol is completed.
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 be contain trace amount magnetic beads. The beads have no influence to proteins and can easily removed through spin-down.*
Note II) *Composition of Storage buffer : [50mM Tris-Cl(pH7.6), 100mM NaCl, 1mM DTT, 0.05% NaN_3, 50% Glycerol].*

Step III Analysis of sample

A. Position of each sample (in Purification Cartridges)



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	20 μL	20 μL

Note) *Bead sample was resuspensed in 500 μL/well sterile distilled water.*

2) Treat the samples at 95 ℃, for 5~10 min.

3) Load 5 μL of each sample to a well of SDS-PAGE gel (10x8(cm), 0.75mm thick, 10 wells).

※ *For more information on how to use this kit, please see our* ***website****.*