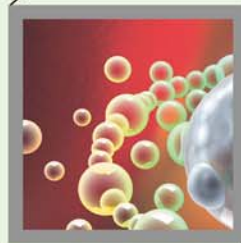




F

**Protein Research**

Protein Expression and Purification  
Protein Size Markers



**Protein Research**

Phone: +82-42-930-8777 Email: [reagents-support@bioneer.com](mailto:reagents-support@bioneer.com)



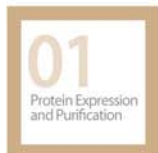
# 01

Protein Expression and Purification

## Protein Expression and Purification



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# Protein Expression and Purification

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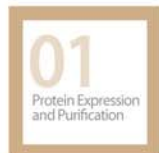
## Overview

With the latest technology and years of experience designing and manufacturing products for protein synthesis and purification, Bioneer is pleased to showcase our Accu- and Exi- series products for protein science. These products are designed to simplify your protein work in a cost-effective way.

At the cutting edge, Bioneer has recently launched the world's first automated protein synthesis/purification and nucleic acid purification system: *ExiProgen*<sup>™</sup>.

*ExiProgen*<sup>™</sup> makes protein synthesis and purification as easy as a thermal cycler makes PCR. In order to achieve this ease of use, we have developed the *ExiProgen*<sup>™</sup> EC Protein Synthesis Kit for cell-free in vitro protein expression and purification. The technology is based on the *AccuRapid*<sup>™</sup> Cell-Free Protein Expression Kit and Ni-NTA magnetic beads used in our *AccuPrep*<sup>®</sup> His-tagged Protein Purification Kit. The *ExiProgen*<sup>™</sup> EC Protein Synthesis Kit is able to express and purify up to 16 unique proteins in parallel in less than 6 hours.

BIONEER's line of protein-related products not only perform standard protein expression and purification, but also provide an automated system on which to perform the tasks, providing the optimal solution needed to enter the Bio 2.0 era.



## AccuRapid™ Cell-Free Protein Expression Kit



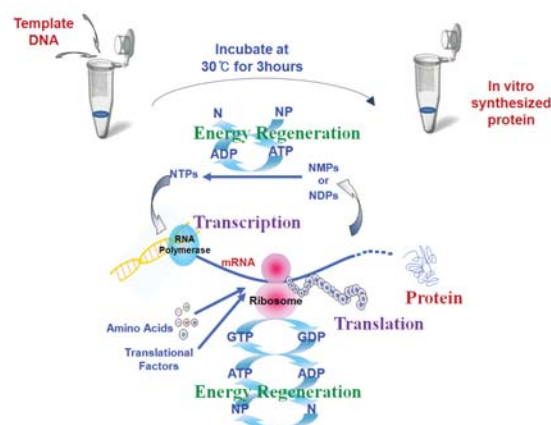
### ■ Description

*AccuRapid™* Cell-Free Protein Expression Kit contains an optimized *E. coli* extract containing T7 RNA polymerase for transcription and all necessary components for translation. When combined with the *AccuRapid™* Master mix supplied in the Kit, all other required components including amino acids, rNTPs, and appropriate salts are provided enabling the expression of high levels of recombinant proteins. This Kit contains reagents for 24 reactions, and each 45  $\mu$ l reaction can express up to 20  $\mu$ g of protein in only 3 hours directly from a variety of DNA templates which contain T7 promoter, T7 terminator and RBS (ribosomal binding site).

### ■ Features and Benefits

- DNA-in-Protein-out simplicity  
Simply add expression vector of PCR products to the Cell-Free Protein Extract/MasterMix.
- Rapid protocol  
The entire protocol is complete in 3 hours!
- Excellent yield  
Each reaction well can yield up to 20  $\mu$ g of protein in only 3 hours.
- Reproducibility  
Minimal pipetting steps to maximize reproducibility.

### ■ Principle of Cell-Free Protein Expression System



### ■ Kit Contents

<i>AccuRapid™</i> Cell-Free Protein Expression Kit (Cat. No. K-7250)	
<i>AccuRapid™</i> Master mix	170 $\mu$ l x 3 tubes (Red Cap)
<i>AccuRapid™</i> <i>E. coli</i> extract	100 $\mu$ l x 3 tubes (Blue Cap)
DNA template	24 $\mu$ l x 1 tube (Green Cap)
DEPC-DW	1 ml x 1 tube (White Cap)

### ■ Storage condition

-20°C (*E. coli* extract; -70°C)

### ■ Experimental Data

#### 1. Protein Expression using *AccuRapid™* Cell-Free Protein Expression Kit

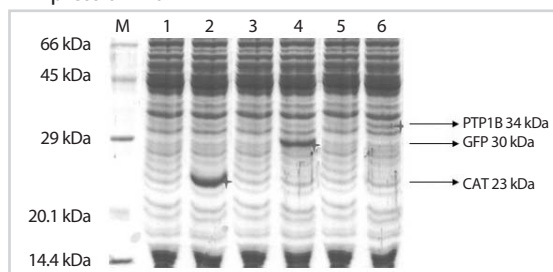


Figure 1. Protein expression Data of *AccuRapid™* Cell-Free Protein Expression Kit (SDS-PAGE and Coomassie Brilliant Blue staining)

Lane M; *AccuLadder™* Protein Size Marker (Low), Lane 1,3,5; Negative control (No-DNA), Lane 2; CAT (Chloramphenicol acetyl transferase), Lane 4; GFP (Green fluorescence protein), Lane 6; PTP1B (Protein tyrosine phosphatase 1B).

#### 2. *AccuRapid™* Cell-Free Protein Expression Kit expresses proteins efficiently

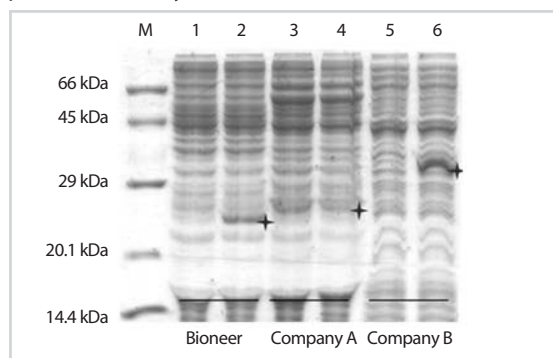


Figure 2. Comparison of protein expression between *AccuRapid™* Cell-Free Protein Expression Kit and protein expression Kits from other companies (SDS-PAGE and Coomassie Brilliant Blue staining).

Lane M; *AccuLadder™* Protein Size Marker (Low), Lane 1,3,5 ; Negative control (No-DNA), Lane 2; Bioneer's Kit and control DNA, Lane 4; Company A's Kit and control DNA, Lane 6; Company B's Kit and control DNA

## AccuRapid™ Cell-Free Protein Expression Kit

3. Determination of the optimal amount of template DNA for cell-free protein synthesis using *AccuRapid*™ Cell-Free Protein Expression Kit

3.1. pBIVT vector construct : GFP

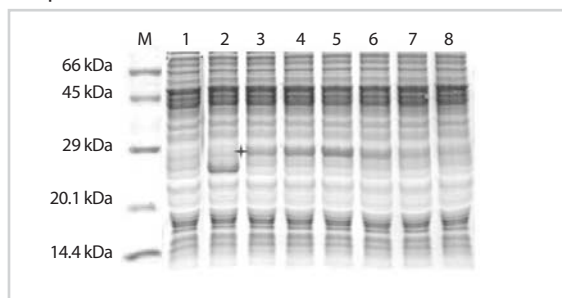


Figure 3. Determination of the optimal amount of template DNA for the expression of GFP using *AccuRapid*™ Cell-Free Protein Expression Kit. GFP was maximally expressed with the *AccuRapid*™ Cell-Free Protein Expression Kit when 200 ng of pBIVT-GFP was used.

Lane M; *AccuLadder*™ Protein Size Marker (Low), Lane 1; Negative Control (No-DNA), Lane 2; CAT (Chloramphenicol acetyl transferase), Lane 3 - 8; pBIVT-GFP (50, 100, 200, 400, 600, and 800 ng per each lane).

3.2. pET vector construct : *MMLV* RTase

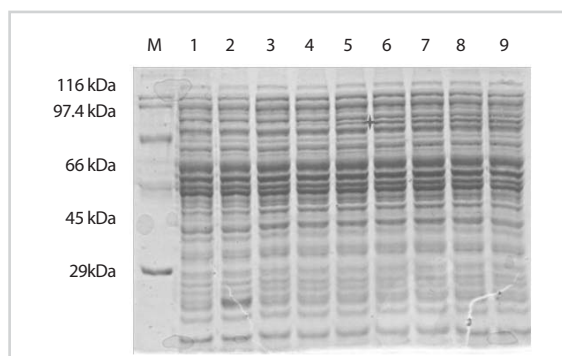
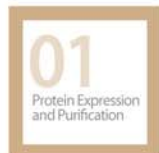


Figure 4. Determination of the optimal amount of template DNA for the expression of *MMLV* RTase using *AccuRapid*™ Cell-Free Protein Expression Kit. *MMLV* RTase was maximally expressed with the *AccuRapid*™ Cell-Free Protein Expression Kit when 600 ng of pET22b-MMLV RTase was used.

M ; *AccuLadder*™ Protein Size Marker (Broad), Lane 1; Negative Control(No-DNA), Lane 2 ; CAT, Lane 3 - 9; pET22b-MMLV RTase (50, 100, 200, 400, 600, 800, and 1000 ng per each lane).

### ■ Ordering Information

Cat. No.	Product Description	O'ty
K-7250	<i>AccuRapid</i> ™ Cell-Free Protein Expression Kit	45 ul x 24 reactions



## AccuPrep<sup>®</sup> His-tagged Protein Purification Kit



### ■ Description

Bioneer's Ni-NTA magnetic silica resins are silica beads, with an average diameter of 1.29  $\mu\text{m}$  and a range of 1~5  $\mu\text{m}$  diameter, have Ni-NTA (nickel-nitrilotriacetic acid) covalently bound to their surface.

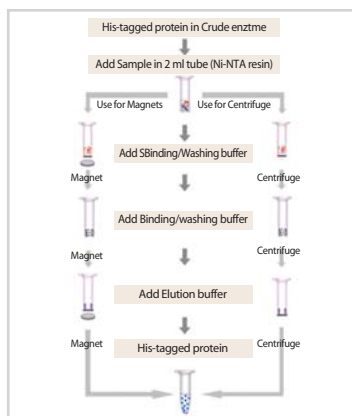
These Ni-NTA magnetic silica beads exhibit outstanding affinity to His-tagged proteins and feature our SSMB (Spherical-Shaped Magnetic Bead) technology that provides increased surface area (due to the small size of the beads), without the issue of impurity carryover common with magnetic particles that have a rough-surface.

Ni-NTA magnetic agarose beads are supplied as a 10% (v/v) suspension with an average binding capacity of 500  $\mu\text{g}$  protein per ml of suspension for 6xHis-tagged protein.

### ■ Features and Benefits

- Flexible: The protocol may be carried out with a magnet or via centrifugation.
- Excellent performance: Highly pure proteins are obtained through our exclusive SSMB (Spherical-Shape Magnetic Beads).
- High purity and High yield: Average binding capacity of 500  $\mu\text{g}$  protein per ml of suspension for 6x His-tagged protein. Purity that is > 90%.
- Reproducibility: The only hands-on step is the addition of protein extract, maximizing reproducibility.
- Fast process: The entire purification is complete in about 30 min!

### ■ Procedure



### ■ Experimental Data

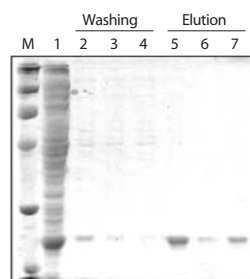


Figure 1. Comparison of purified protein using several purification Kits.

Lane M: Protein Size Marker

Lane 1: Loading Sample

Lane 2, 5: Purified fragment using AccuPrep<sup>®</sup> His-tagged Protein Purification Kit from Bioneer

Lane 3, 6: Purified fragment using Protein Purification Kit from Company Q

Lane 4, 7: Purified fragment using Protein Purification Kit from Company E

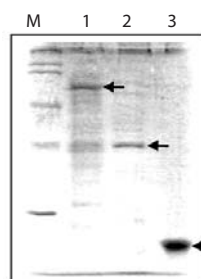


Figure 2. Purified proteins using AccuPrep<sup>®</sup> His-tagged Protein Purification Kit

Lane M: Protein Size Marker

Lane 1: TLA DNA Polymerase

Lane 2: PTPase #54

Lane 3: PTPase pk6

## AccuPrep<sup>®</sup> His-tagged Protein Purification Kit

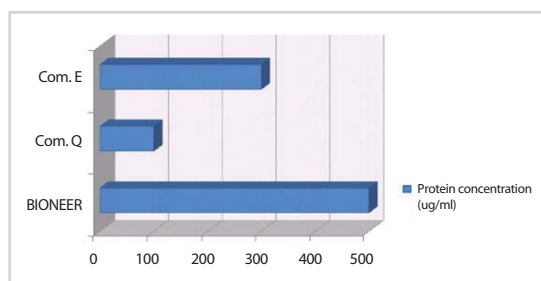
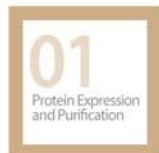


Figure 3. Concentration of TLA DNA polymerase protein using several protein purification Kits.

### ■ Ordering information

Cat. No.	Product Description
K-7200	AccuPrep <sup>®</sup> His-tagged Protein Purification Kit 5 X 1 mL (10% suspension)



## ExiPrep™ His-tagged Protein Purification Kit

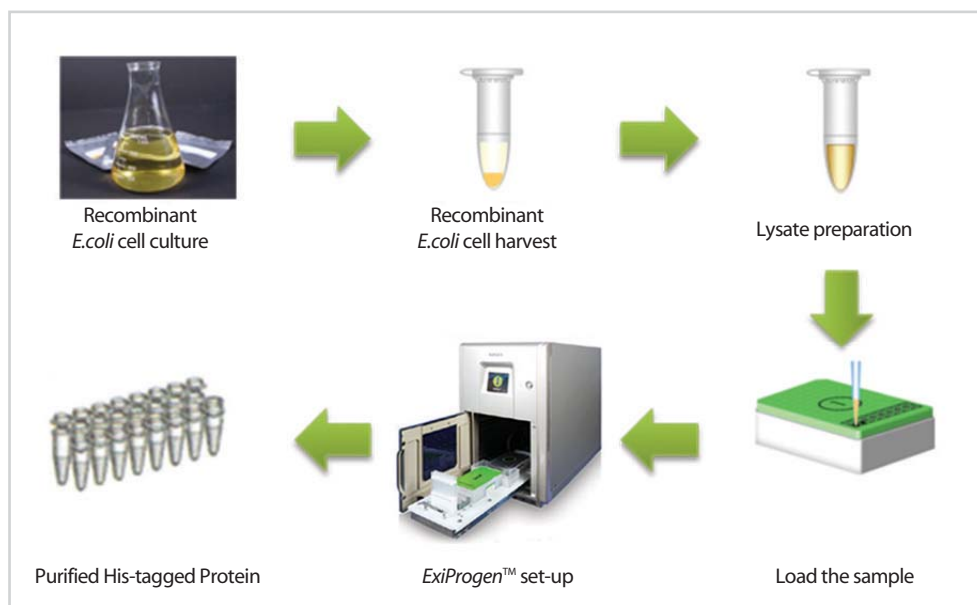


### ■ Description

*ExiPrep™* His-tagged Protein Purification Kit is suitable to extract of Histidine-tagged protein from recombinant *E. coli*, by automatic instrument *ExiPrep™* 16 series.

Ni-NTA Magnetic Silica Resins are silica beads, with an average diameter of 1~5  $\mu\text{m}$ , that contain magnetic particles and have strongly metal-chelating nitrilotriacetic acid (NTA) groups covalently bound to their surfaces.

### ■ Procedure



### ■ Features and Benefits

- Automatic purification of His-tagged proteins
- Getting highly pure proteins through SSMB(Spherical Shape Magnetic Beads)
- Reproducible result by minimizing human handling
- Finishing whole process within 30 minutes

### ■ Kit Component and Protocol

*ExiProgen™* His-tagged Protein Purification Kit  
(Cat. No. K-7210)

Cartridge	6 ea
Disposable tip	3 packs (32 ea / 1 pack)
Elution tube	96 ea / 1 pack
Resuspension buffer	50 ml X 1 ea



# ExiPrep™ His-tagged Protein Purification Kit

## ■ Experimental Data

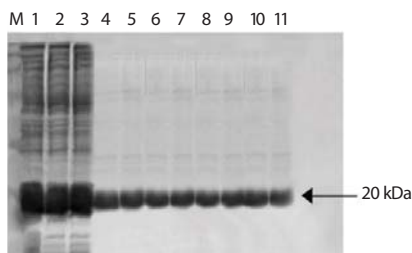


Figure 1. Electrophoresis was performed on a vertical gel containing 13% poly-acrylamide.

Lane 1: crude extract of gram negative cell by sonication  
Lane 2 - 3: unbound protein (duplication)  
Lane 4 - 11: purified PTPase #32

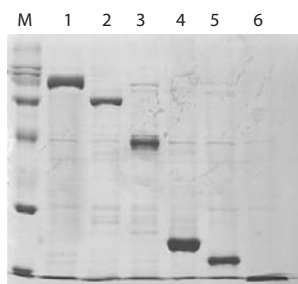


Figure 2. The purification was performed using *ExiProgen*™, and samples were electrophoresed on a 10% polyacrylamide gel.

Lane M: Protein size marker (Broad, Bioneer)  
Lane 1: Purified protein of 92 kDa  
Lane 2: Purified protein of 65 kDa.  
Lane 3: Purified protein of 44 kDa.  
Lane 4: Purified protein of 25 kDa.  
Lane 5: Purified protein of 22 kDa.  
Lane 6: Purified protein of 15 kDa

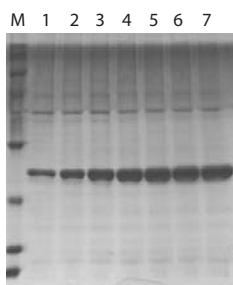
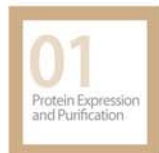


Figure 3. Target protein is 25 kDa protein for tagging of Histidine. The purification was performed using *ExiProgen*™, and samples were electrophoresed on a 13% polyacrylamide gel.

Lane M: Protein Size Marker (Broad, Bioneer, Cat. No.D-2010)  
Lane 1: purified sample of 0.8 mg/mL  
Lane 2: purified sample of 1.6 mg/mL  
Lane 3: purified sample of 3.2 mg/mL  
Lane 4: purified sample of 6.4 mg/mL  
Lane 5: purified sample of 9.8 mg/mL  
Lane 6: purified sample of 13.0 mg/mL  
Lane 7: purified sample of 16.2 mg/mL

## ■ Ordering Information

Cat No.	Product Description
K-7210	<i>ExiPrep</i> ™ His-tagged Protein Purification Kit, 96 preps
K-7220	<i>ExiProgen</i> ™ His-tagged Protein Purification Kit, 96 preps
K-7221	<i>ExiProgen</i> ™ His-tagged Protein Purification Kit 32 preps



## ExiProgen™ EC Protein Synthesis Kit

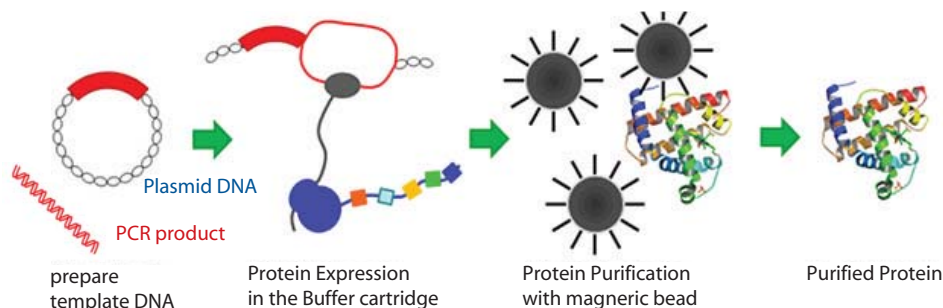
### ■ Description



Bioneer's ExiProgen™ EC Protein Synthesis Kit is designed for *in vitro* (cell-free) expression and high-yield Histidine-Tag affinity purification of proteins by using the world's first automated protein synthesis and nucleic acid extraction system "ExiProgen™". 1 to 16 different types of highly pure proteins can be obtained from each run. Each reaction needs only 6-10 µg of DNA, which can be in the form of an expression vector such as Bioneer's pBIVT or a PCR product using the ExiProgen™ ProXpress PCR Template Kit. Because the entire process is automated, you will obtain reliable and reproducible results run after run

### ■ Principle of Protein Synthesis and Purification

ExiProgen™ EC Protein Synthesis Kit contains optimized reagents for efficient expression of various protein types, and also contains reagents and Ni-NTA magnetic beads for purification of expressed proteins. Target proteins synthesized from recombinant plasmid DNAs are bound to Ni-NTA magnetic beads and washed with washing buffer for highly pure proteins.



### ■ Features and Benefits

- Fully automatic: DNA-in-Protein-out  
Input 6~10 µg of template DNA and load the Kit into ExiProgen™ for high-purity proteins within 6 hrs.
- Parallel Process  
Obtain from 1 to 16 different proteins in a single run.
- High performance  
The system allows for synthesis of proteins that are toxic to *in vivo* expression systems.
- High purity and High yield  
Each reaction well will yield up to 100 µg protein that is over 90 % pure.
- Reproducibility  
The only hands-on step is the addition of template DNA, maximizing reproducibility.
- Fast process  
The *in vitro* (cell-free) protein synthesis system does not require a time-consuming cell culture process.

### ■ Kit Contents and Storage conditions

	Components	Storage condition
Kit-1 (Purification Kit)	Cartridge ①	4 °C
	Disposable filter tip	Room temperature
	Manual	
Kit-2 (Expression Kit)	Cartridge ②	-20 °C
	Positive control DNA 1.5ml tube	-20 °C
	<i>E. coli</i> Cell extract 8 strip tube	-70 °C
	Elution tube and cap	Room temperature

# ExiProgen™ EC Protein Synthesis Kit

## ■ Experimental Data

**1. Reproducibility:** ExiProgen™ EC Protein Synthesis Kits provide reproducible results, the first time and every time.

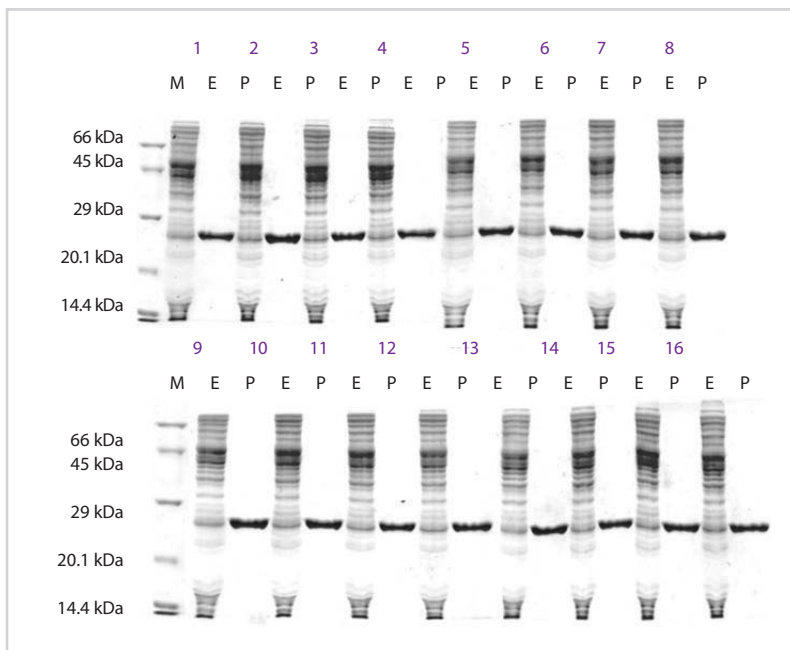


Figure 1. Expression and purification of CAT. Reproducibility is seen in all 16 wells with no detectable variation between wells. Lane 1~16: Number of the well, M: AccuLadder™ Protein Size Marker (Low), E : Expression sample, P : Purification sample

**2. Flexibility:** Proteins as small as 10 kDa and as large as 120 kDa have been expressed/purified with ExiProgen™.

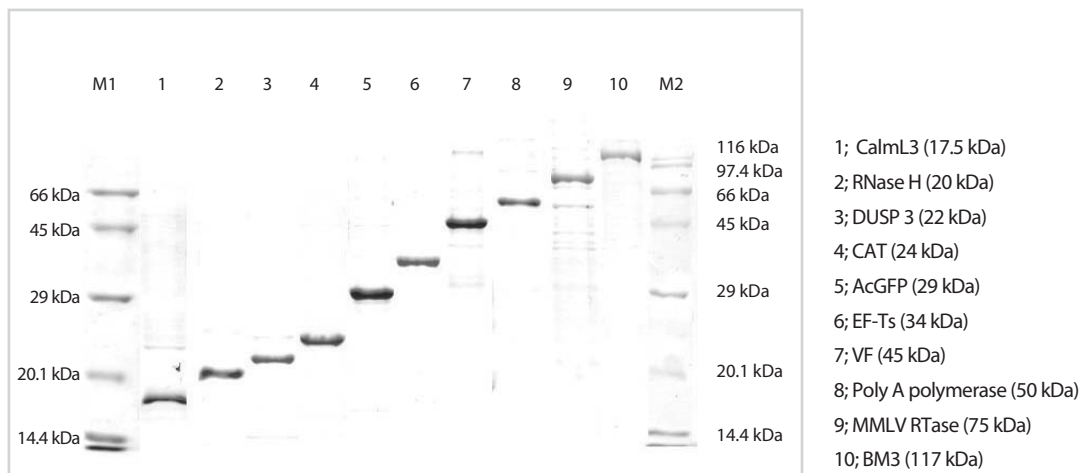
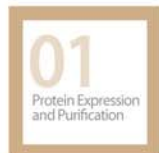


Figure 2. Expression and purification of various proteins. Up to 16 types of proteins can be expressed and purified simultaneously with an average of over 90% purity.

M1: AccuLadder™ Protein Size Marker (Low), M2: AccuLadder™ Protein Size Marker (Broad)



## ExiProgen™ EC Protein Synthesis Kit

**3. Control:** Each Kit comes with a Green Fluorescent Protein positive control providing confidence in the results

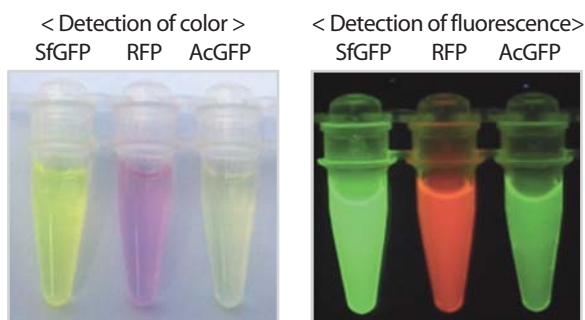


Figure 3. Detection of fluorescence emitted from fluorescent proteins synthesized with *ExiProgen*™. This result indicates that these synthesized SfGFP, RFP, and AcGFP are functionally active.

Left panel: Color of each protein elution samples detected with naked eyes.

Right panel: Fluorescence from protein elution samples detected with UV illuminator.

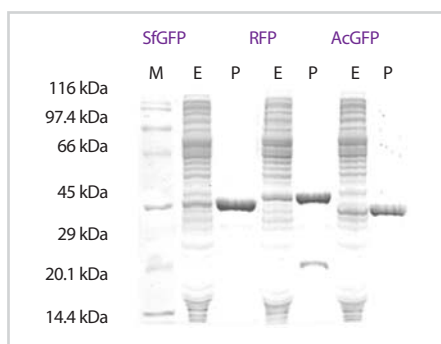


Figure 4. Expression and purification of fluorescent proteins.

Top of the lane: Protein name, Lane M: *AccuLadder*™ Protein Size Marker (Broad), Lane E: Expression sample, Lane P: Purification sample

**4. Functionality:** Proteins expressed/purified with *ExiProgen*™ can be assayed for functionality and the resulting proteins will generally yield up to 100 µg or > 90% pure protein. Many established recombinant proteins can be expressed in *E. coli* extracts with full functional activity.

< Activity check >

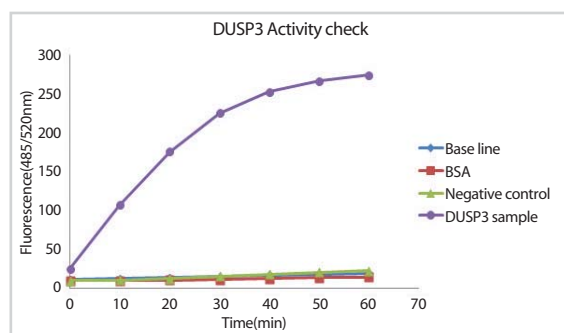
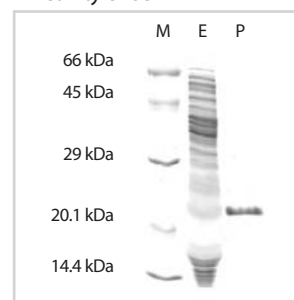


Figure 5. DUSP3 synthesized with *ExiProgen*™ has an enzyme activity.

Panel A : Expression and purification of DUSP3 (Protein tyrosine phosphatase).

M : *AccuLadder*™ Protein Size Marker (Low), Lane E : Expression sample, Lane P : Purification sample.

Panel B : Activity check of DUSP3 (Protein tyrosine phosphatase).

DUSP3 phosphatase activity was measured by incubating with 500 µM 3-O-methylfluorescein phosphate.

(OMFP). (Reaction buffer : 100 mM Tris-HCl (pH8.2), 40 mM NaCl, 1 mM DTT, and 20 % Glycerol).

### Ordering information

Cat. No.	Product Description
K-7300	<i>ExiProgen</i> ™ EC Protein Synthesis Kit, 16 reactions
K-7301	<i>ExiProgen</i> ™ EC Protein Synthesis Kit, 32 reactions
K-7302	<i>ExiProgen</i> ™ EC Protein Synthesis Kit, 96 reactions



# 02

Protein Size Markers

## Protein Size Markers

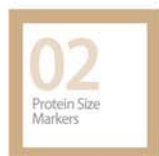


Bioneer Corporation



<i>AccuLadder</i> <sup>™</sup> Protein Size Marker (Broad) .....	254
<i>AccuLadder</i> <sup>™</sup> Protein Size Marker (Low) .....	255

Protein Size Markers



## AccuLadder™ Protein Size Marker (Broad)



### ■ Description

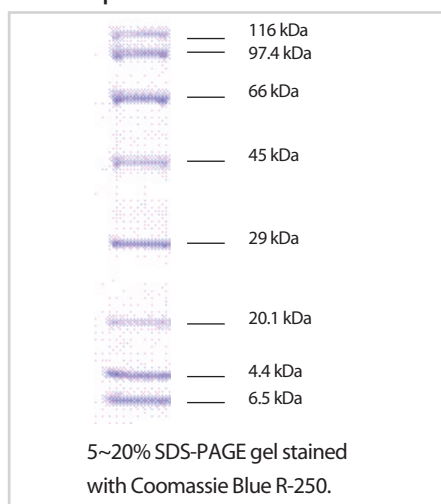
AccuLadder™ Protein Size Marker (Broad) consists of 8 kinds of proteins (6.5 – 116 kDa).

This Protein size marker contains sample buffer, and it can be loading directly after heating at 95°C for 5 minutes. 5 µl of marker is sufficient to be loaded per lane of SDS-PAGE minigel, and one vial is for 100 lanes in this case.

### ■ Features and Benefits

- Concentration: 0.1 - 0.2 mg/ml
- Recommended loading: 5 µl/lane (Minigel ; 10x8(cm<sup>2</sup>), 0.75 or 1.0mm thick)
- Size Range (kDa): 6.5 ~ 116
- Number of Bands: 8

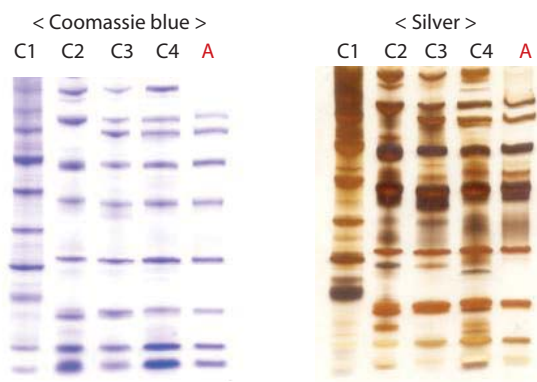
### ■ Components



Protein	MW (kDa)	Source
β-Galactosidase	116	<i>E. coli</i>
Phosphorylase b	97.4	Rabbit muscle
Albumin	66	Bovine serum
Ovalbumin	45	Chicken egg white
Carbonic anhydrase	29	Bovine erythrocytes
Trypsin inhibitor	20.1	Soybean
Lysozyme	14.4	Chicken egg white
Aprotinin	6.5	Bovine lung

Note: Do heat before loading (95°C, 5 min) for each use. Repeated freezing and thawing should be avoided.

### ■ Experimental data



C 1; Company 1, C 2; Company 2, C 3; Company 3, C 4; Company 4

A; AccuLadder™ Protein Size Marker (Broad)

### ■ Ordering Information

Cat. No.	Product Description
D-2010	AccuLadder™ Protein Size Marker (Broad), 500 µl

## AccuLadder™ Protein Size Marker (Low)



### ■ Description

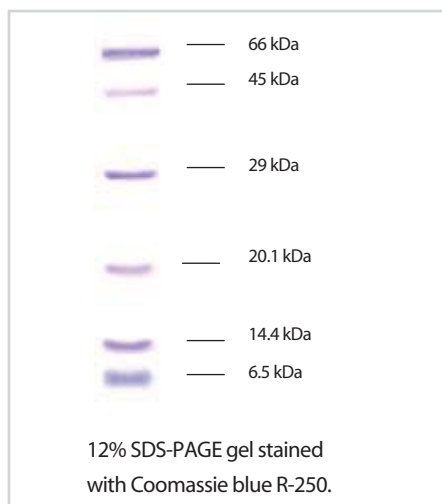
AccuLadder™ Protein Size Marker (Low) consists of 6 kinds of proteins (6.5 – 66 kDa).

This Protein size marker contains sample buffer, and it can be loading directly after heating at 95°C for 5 minutes. 5 µl of marker is sufficient to be loaded per lane of SDS-PAGE minigel, and one vial is for 100 lanes in this case.

### ■ Features and Benefits

- Concentration: 0.1 - 0.2 mg/ml
- Recommended loading: 5 µl/lane (Minigel ; 10x8(cm<sup>2</sup>), 0.75 or 1.0mm thick)
- Size Range (kDa): 6.5 ~ 66
- Number of Bands: 6

### ■ Components



Protein	MW (kDa)	Source
Albumin	66	Bovine serum
Ovalbumin	45	Chicken egg white
Carbonic anhydrase	29	Bovine erythrocytes
Trypsin inhibitor	20.1	Soybean
Lysozyme	14.4	Chicken egg white
Aprotinin	6.5	Bovine lung

Note: Do heat before loading (95°C, 5 min) for each use. Repeated freezing and thawing should be avoided.

### ■ Ordering Information

Cat. No.	Product Description
D-2020	AccuLadder™ Protein Size Marker (Low), 500 µl

02  
Protein Size  
Markers

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