Profinia™ Bacterial Lysis/Extraction Reagent

Catalog Number
620-0220

For technical support, call your local Bio-Rad office or, in the US, call
1-800-4BIORAD (1-800-424-6723)

Catalog Number: 620-0220

Volume: 250 ml

Storage Conditions: Room temperature

Introduction

Profinia bacterial lysis/extraction reagent was developed for the lysis and extraction of recombinant proteins from *Escherichia coli*. The reagent is formulated using a sodium phosphate-based buffer, pH 7.5, and includes a proprietary mild nonionic detergent for the lysis of bacterial cells. Lysis of cultures using the Profinia extraction reagent can be more reproducible and less technically challenging than traditional mechanical lysis methods such as sonication. Additional components such as nucleases, protease inhibitors, salts, reducing agents, chelating agents, or other standard additives may be added to the reagent to customize individual protocols and proteins. The Profinia bacterial lysis/extraction reagent may be used for both soluble protein extraction and inclusion body purification from complex bacterial cell lysates.

General Information

1. **Soluble Proteins and Inclusion Bodies**: Recombinant proteins overexpressed in *E. coli* can partition into the soluble fraction, into the insoluble inclusion body fraction, or can partially fractionate into both fractions. The inclusion body fraction contains a very high percentage of overexpressed target protein, and isolation of the inclusion bodies and subsequent solubilization and purification of target proteins from this fraction provides an efficient first step of enhancement. Profinia bacterial lysis/extraction reagent effectively extracts soluble proteins and separates most of the soluble protein from the inclusion bodies. It is usually advised to perform a small-scale expression and extraction to determine the solubility of the specific recombinant protein before performing a larger-scale protein extraction and purification.

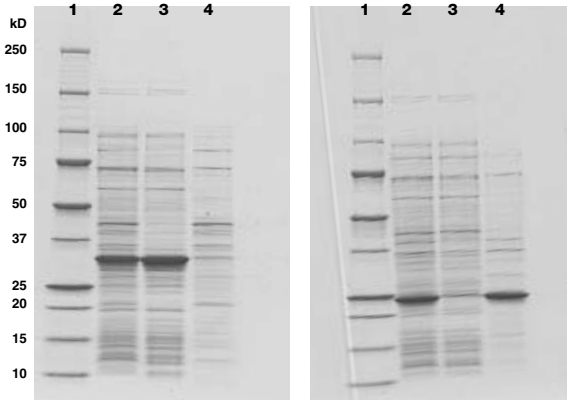
2. **Fresh Cells and Frozen Cells**: Profinia bacterial lysis/extraction reagent can be used to extract proteins from both fresh and frozen cells. Freezing cell pellets after induction and centrifugation is a convenient stopping point and proteins are extracted from frozen pellets more effectively than freshly prepared pellets.

3. ***E. coli* Strain**: Profinia bacterial lysis/extraction reagent can be used with several common bacterial host strains. It is especially suitable for the commonly used protease-deficient bacterial expression host BL21 strains.

4. **Compatibility**: Profinia bacterial lysis/extraction reagent is supplied in a phosphate-based buffer system. Phosphate buffer is recommended for protein purification following protein extraction. The buffers in the Profinia IMAC purification kits utilize phosphate buffers and complement the Profinia bacterial lysis/extraction reagent.

Sample Preparation Protocols

The following protocol for Profinia bacterial lysis/extraction reagent can be scaled for any size culture, but has been optimized for cultures between 100 ml and 1,000 ml (OD_{600} = 1.5–4.0). Before full-scale cultures and extractions are performed, the soluble and insoluble fractions should be examined from ~1 ml *E. coli* cultures in order to know which protocol to follow. Figure 1 illustrates representative total, soluble, and insoluble (lanes 2–4) fractionation screening for *E. coli* cultures expressing 32 and 23 kD proteins, respectively.



~30% expression
Soluble partitioning

~25% expression
Insoluble partitioning

Fig. 1. Partitioning profiles. For both gels, Precision Plus Protein™ molecular weight markers were loaded in lane 1, followed by the total, soluble, and insoluble fractions in lanes 2–4, respectively. The first panel depicts a 32 kD target protein, which partitions into the soluble fraction and can be purified using the native protocol. The second panel depicts a 23 kD target protein, which partitions into the insoluble fraction and can be purified using the denaturing protocol.

For *E. coli* cultures expressing medium to high levels of fusion proteins (≥10% of total protein), 200 ml of culture will normally yield sufficient material for a 1 ml cartridge purification, and 1,000 ml of culture will yield sufficient material for a 5 ml cartridge purification. For cultures expressing protein at low levels (≤10% of total protein), the culture volumes will need to be empirically determined for each protein. Bacterial cultures can be grown in advance and centrifuged. The pellets can be stored at –70°C for several months and lysed at a convenient date for sample preparation.

Procedure for Purifying Soluble Proteins

1. Pellet bacterial cells by centrifugation at 6,000 x g for 10 min at 4°C. The pellet can be processed as a fresh pellet, or frozen at –70°C and lysed at a later date.
2. Determine the weight of the bacterial cell pellet and resuspend in 10 volumes of Profinia bacterial lysis/extraction reagent (1:10 w/v). Thoroughly resuspend the pellet by pipetting or vortexing.

   Note: 200 ml of culture will typically yield 0.8–1.0 g of paste, or 8–10 ml of lysate.
3. To minimize lysate viscosity, a nuclease (such as Benzonase) can be added to the resuspended pellet. Add Benzonase at 25 units/ml (or DNAse at 100 units/ml), and incubate for 10 min at room temperature.

Note: Solutions that have high viscosity can create backpressure during subsequent chromatographic purifications.

4. Separate the soluble from the insoluble proteins by centrifugation at 16,000 x g for 20 min at 4°C. The supernatant will contain the soluble extract and soluble target proteins, and the insoluble pellet can be discarded.
5. Remove the supernatant containing the soluble extract and filter through a 0.45 µm filter to remove particulates. The filtrate is now ready for purification on the Profinia system. Keep the lysate on ice until ready to proceed.

   Note: The lysate can be frozen and purified at a later date. However, proteolysis or protein degradation can occur upon freezing and thawing, and the quality of the purified product may be compromised. Upon thawing, refilter the lysate through a 0.45 µm filter, as precipitates often form after freezing.

Procedure for Purifying Insoluble Proteins From Inclusion Bodies

1. Pellet bacterial cells by centrifugation at 6,000 x g for 10 min at 4°C. The pellet can be processed as a fresh pellet, or frozen at –70°C and lysed at a later date.
2. Determine the weight of the bacterial cell pellet and resuspend in 10 volumes of Profinia bacterial lysis/extraction reagent (1:10 w/v). Thoroughly resuspend the pellet by pipetting or vortexing.

   Note: 200 ml of culture will typically yield 0.8–1.0 g of paste, or 8–10 ml of lysate.
3. To minimize lysate viscosity, a nuclease (such as Benzonase) can be added to the resuspended pellet. Add Benzonase at 25 units/ml (or DNAse at 100 units/ml), and incubate for 10 min at room temperature.

   Note: Solutions that have high viscosity can create backpressure during subsequent chromatographic purifications.
4. Separate the soluble from the insoluble proteins by centrifugation at 16,000 x g for 20 min at 4°C.
5. Discard the supernatant and add 10 volumes of Profinia denaturing IMAC wash buffer 1 (or a comparable solution containing 6 M urea) to the inclusion body pellet. Vortex or pipet well to thoroughly resuspend the pellet.
6. Centrifuge the lysate at 16,000 x g for 20 min at 4°C.
7. Remove the supernatant containing the solubilized pellet and filter through a 0.45 µm filter to remove particulates. The filtered lysate is now ready for purification on the Profinia system. Keep the lysate on ice until ready to proceed.

   Note: The lysate can be frozen and purified at a later date. However, proteolysis or protein degradation can occur upon freezing and thawing, and the quality of the purified product may be compromised. Upon thawing, refilter the lysate through a 0.45 µm filter, as precipitates often form after freezing.

Ordering Information

Catalog #	Description
Profinia Instruments	
620-1004	Profinia Instrument With Accessory Kit, 100–240 V
620-1005	Profinia Instrument With Accessory Kit and Native IMAC Starter Kit, 100–240 V
620-1006	Profinia Instrument With Accessory Kit and GST Starter Kit, 100–240 V
Profinia Systems	
620-1009	Profinia System, 100–240 V, includes accessory kit and Profinia software
620-1010	Profinia System With Native GST Starter Kit, 100–240 V
620-1011	Profinia System With GST Starter Kit, 100–240 V
Profinia Accessories	
620-0010	Profinia Software With USB Cable
620-0401	Profinia Cooling Accessory
620-0402	Profinia 2.0 ml Desalting Sample Loop
620-0403	Profinia 10.0 ml Desalting Sample Loop
620-0404	Profinia Instrument Inline Filter Assembly Replacement
620-0410	Profinia Instrument Accessory Kit, includes cleaning tray, inline filter assembly replacement, 8 buffer lids, 2 x 50 ml sample lids, 2 x 15 ml lids, bottle starter pack, waste/diluent bottle set
620-0231	Bottle Starter Pack, 4 x 125 ml and 4 x 250 ml bottles and 8 buffer lids
620-0411	Profinia pH Monitor With Mounting Accessory Kit, includes pH electrode, flow cell, mounting accessories
Profinia Purification Kits	
620-0225	Profinia Native IMAC Purification Kit, 1 ml, includes Profinia native IMAC buffer kit, 2 x 1 ml IMAC and 2 x 10 ml desalting cartridges
620-0235	Profinia Native IMAC Purification Kit, 5 ml, includes 2 Profinia native IMAC buffer kits, 1 x 5 ml IMAC and 1 x 50 ml desalting cartridge
620-0227	Profinia Denaturing IMAC Purification Kit, 1 ml, includes Profinia denaturing IMAC buffer kit, 2 x 1 ml IMAC cartridges
620-0237	Profinia Denaturing IMAC Purification Kit, 5 ml, includes 2 Profinia denaturing IMAC buffer kits, 2, 2 x 1 ml IMAC cartridges
620-0226	Profinia GST Purification Kit, 1 ml, includes Profinia GST buffer kit, 2 x 1 ml GST and 2 x 10 ml desalting cartridges

Catalog #	Description
620-0236	Profinia GST Purification Kit, 5 ml, includes 2 Profinia GST buffer kits, 1 x 5 ml GST and 1 x 50 ml desalting cartridge
620-0228	Profinia Desalting Purification Kit, 10 ml, includes Profinia desalting buffer kit, 2 x 10 ml desalting cartridges
620-0238	Profinia Desalting Purification Kit, 50 ml, includes 2 Profinia desalting buffer kits, 1 x 50 ml desalting cartridge

Profinia Buffer Kits

620-0221	Profinia Native IMAC Buffer Kit, includes purification buffers, cleaning and storage solutions; sufficient for 10 applications
620-0222	Profinia Denaturing IMAC Buffer Kit, includes purification buffers, cleaning and storage solutions, urea reagent; sufficient for 10 applications
620-0223	Profinia GST Buffer Kit, includes purification buffers, cleaning and storage solutions, glutathione reagent; sufficient for 10 applications
620-0224	Profinia Desalting Buffer Kit, includes purification buffers, cleaning and storage solutions; sufficient for 10 applications

Profinia Starter Kits

620-0229	Profinia IMAC Starter Kit, includes Profinia native IMAC buffer kit, 1 x 1 ml IMAC and 1 x 10 ml desalting cartridge, *E. coli* lysate
620-0230	Profinia GST Starter Kit, includes Profinia GST buffer kit, 1 x 1 ml GST and 1 x 10 ml desalting cartridge, *E. coli* lysate, glutathione reagent

Profinia Reagents

620-0220	Profinia Bacterial Lysis/Extraction Reagent
620-0203	Profinia His Antibody
620-0204	Profinia GST Antibody
620-0233	Profinia Control Lysate
620-0200	Profinia Small Urea Pack, 45 g
620-0201	Profinia Large Urea Pack, 90 g
620-0202	Profinia Glutathione Pack, 1.23 g

Catalog #	Description

Bio-Scale Mini Affinity and Desalting Cartridges

732-4610	Bio-Scale Mini Profinity IMAC Cartridges, 5 x 1 ml
732-4612	Bio-Scale Mini Profinity IMAC Cartridge, 1 x 5 ml
732-4614	Bio-Scale Mini Profinity IMAC Cartridges, 5 x 5 ml
732-4620	Bio-Scale Mini Profinity GST Cartridges, 5 x 1 ml
732-4622	Bio-Scale Mini Profinity GST Cartridge, 1 x 5 ml
732-4624	Bio-Scale Mini Profinity GST Cartridges, 5 x 5 ml
732-5304	Bio-Scale Mini Bio-Gel P6 Desalting Cartridges, 5 x 10 ml
732-5312	Bio-Scale Mini Bio-Gel P6 Desalting Cartridge, 1 x 50 ml
732-5314	Bio-Scale Mini Bio-Gel P6 Desalting Cartridges, 5 x 50 ml

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