ISO 9001/14001 Certified Company

SMART™ Bacterial Protein Extraction Solution

Cat. No. 17511 **110ml**

DESCRIPTION

The SMART™ Bacterial Protein Extraction Solution is designed for fast and easy extraction of total proteins from bacteria without the need for sonication or precipitation. This solution utilizes a proprietary non-ionic detergent in 20mM Tris/HCl(pH7.5). In addition, the SMART™ Bacterial Protein Extraction Solution offers several folding increase in the yield of soluble protein when compared with other commercial lysis reagent. Depending on the particular application, additional components, such as lysozyme, protease inhibitors, salts, reducing agents and chelating agents may be added to the solution. The solution may be used for both soluble protein extraction and inclusion body purification from bacterial cell lysates. In fact, yields obtained with SMART™ Bacterial Protein Extraction Solution greatly exceeds those obtained using standard sonication methods!

Bacteria often over-express recombinant proteins and form inclusion bodies, which are insoluble aggregates of misfolded protein. Centrifugation separates inclusion bodies from soluble proteins; however, Lysozyme is required for purification of inclusion bodies. Lysozyme significantly improves inclusion body purity by digesting the cell debris.

CHARACTERISTICS

• Non-ionic detergent in a Tris/HCl (pH7.5) buffer. Therefore, use Tris buffer for subsequent protein purification. Eliminates mechanical disruption and provides a gentle and efficient method for extracting proteins.

• Whole cell lysates prepared with the SMART™ Bacterial Protein Extraction Solution are compatible with the SMART™ BCA Protein Assay Kit(Cat.No.21071) and coomassie protein assays.

KIT CONTENTS

• SMART™ Bacterial Protein Extraction Solution	110 ml

STORAGE

• Upon receipt store product at room temperature.

Note

Assay the extraction by analyzing 20µl aliquots by electrophoresis through a 10% SDS-PAGE and store the fraction containing target protein at -20°C or directly load onto the purification

GUIDELINE

• Protease inhibitors

- Protease inhibitors were not supplied in SMART™ Bacterial Protein Extraction Solution.
- Protease inhibitors may be added, but do not add EDTA or other chelating agents when use Ni^{2+} affinity column, for the chelators destroy the column's ability to bind histidine.

• Use of lysozyme

- Although SMART™ Bacterial Protein Extraction Solution may be used conjunctively with lysozyme, it is not necessary to extract soluble proteins. However, when isolating inclusion bodies, lysozyme should be used to digest cell debris to release inclusion bodies.

• PMSF is dissolved in isopropanol and should be stored at -20°C

- Operate carefully PMSF, which is extremely toxic and irritative to eyes and skin. Wear eye and hand protection to keep the solution containing PMSF away for your safety. This product is for laboratory research use only.

Protease Inhibitor	Inhibitive protease	Concentration
Benzamide (M.W=174.6)	Serine Protease	0.5~5 mM
PMSF (M.W=174.2)	Serine Protease	0.1~10 mM
Antipain (M.W=604.7)	Cysteine Protease	1~10 µg/ml
Chymostatin (M.W=607.7)	Chemotrypsin	1~10 µg/ml
Leupeptin (M.W=426.6)	Serine, Cysteine Protease	1~10 µg/ml
Peptatin A (M.W=685.9)	Asparagin acid Protease	1~10 µg/ml
Aprotinin (M.W=6,500)	Kallikrein, Trypsin, Chemotrypsin	1~50 µg/ml
EDTA (M.W=372.24)	Metal protease	1~10 mM

PROTOCOL 1 (Soluble Protein Extraction)

1. Harvest cells from 1.5 ml bacterial culture (OD_{600} 1.5 ~ 3.0) at 10,000 rpm for 10 minutes in a microcentrifuge.
   NOTE : For larger volumes, e.g. 40~250 ml of bacterial culture, pellet cells by centrifugation at 13,000 rpm for 15 minutes.
2. Remove all media by aspiration. The cells can either be used fresh or frozen at – 80°C.
3. Resuspend cells in 350 µl of SMART™ Total Protein Extraction Solution by vigorously vortexing sample for 1 minute.
   NOTE : If the pellet was harvested from 40~250 ml of culture, resuspend in 8 ~ 25 ml of SMART™ Bacterial Protein Extraction Solution should be used .
   If BL21(DE3)pLysS strain is used, add 1000 U/ml of DNase I in this step.
   Protease inhibitors cocktails may be added this step.
4. Centrifuge at 13,000 rpm for 5 minutes to separate the soluble and insoluble fractions. The soluble protein is in the supernatant.
   NOTE : For larger volumes, separation of soluble and insoluble fractions can be achieved by centrifugation at 13,000 rpm for 20 minutes.
5. Transfer the supernatant to a clean tube and resuspend the insoluble fraction in 350 µl of SMART™ Bacterial Protein Extraction Solution. Use 10 µl each of the soluble and insoluble fraction for SDS-PAGE and/or Western blotting to determine the solubility of the recombinant protein of interest.

PROTOCOL 2 (Inclusion Body Purification)

6. Follow steps 1 through 5 above for soluble protein extraction.
7. Add lysozyme to the resuspended pellet to a final concentration of 400 µg/ml. Vortex for 1 minute and incubate at room temperature for 5 minutes..
8. Add 1 ml of 1:10 diluted SMART™ Bacterial Protein Extraction Solution (with DW) to suspend and vortex for 1 minute.
9. Centrifuge at 13,000 rpm for 10 minutes. Resuspend pellet in 1 ml of 1:10 diluted SMART™ Bacterial Protein Extraction Solution (with DW). Vortex for 1minute. Remove supernatant with pipette. Repeat this step two more times.
   NOTE : For larger volumes, separation of inclusion bodies can be achieved by centrifugation at 13,000 rpm for 20 minutes.
   Step 4 and 5 may be repeated for multiple rounds of purification if necessary.
10. Resuspend the pellet of the purified inclusion bodies in the buffer of your choice. Use 10~20 µl of sample for SDS-PAGE to determine purity.

EXPERIMENTAL DATA

Comparison of Protein Recovery with Competitor

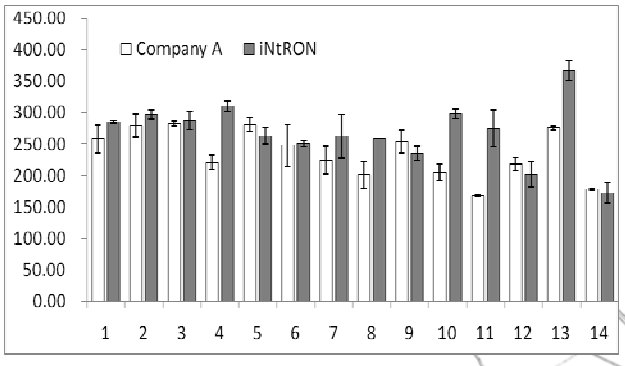

Fig. 1. Comparison of efficiency of protein extraction with competitor's Kit. The results of Table 1 was demonstrated graphically. The SMART™ Bacterial Protein Extraction Solution showed improved recovery of bacterial protein extraction.

ADDITIONAL INFORMATION

Table. 1. Comparison of extracted protein concentration with competitor
The quantities of extracted proteins were estimated using by SMART™ BCA Protein Assay Kit (Cat.No 21071)

Sample ID	Company A Concentration (μg/ml)	iNtRON Concentration (μg/ml)
1: *Escherichia coli*	257.72	285.28
2: *Enterobacter aerogenes*	279.19	297.04
3: *Shigella dysenteriae*	283.08	287.52
4: *Pseudomonas aeruginosa*	220.96	310.37
5: *Streptococcus aureus*	280.93	263.05
6: *Corynebacterium sp.*	247.82	250.64
7: TOP 10	224.30	262.16
8: DH 5α	201.36	257.81
9: BL21(DE3)	253.61	234.72
10: JM 109	205.44	298.05
11: *M15*	168.25	274.87
12: *origami*	218.02	201.67
13: *BL21*	275.57	367.06
14: *Rosettagami*	177.93	171.65

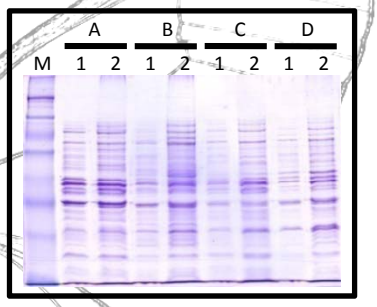

Fig. 2. SDS-PAGE Analysis
Each protein extracted with different bacterial protein extraction kits was analyzed on SDS-PAGE Gel.
The SMART™ Bacterial Protein Extraction Solution (lane 2) shows improved extraction efficacy better than competitor's Kit (lane 1).
Lane A, M15(pRep4); lane B, Origami; lane C, BL21(DE3); lane D, Rogettagami; lane M, PRO-STAIN™ (I) Prestained Protein Marker (cat. No. 24051)

Western Blotting Analysis

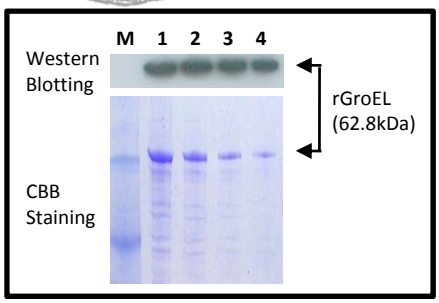

Fig. 3. SDS-PAGE & Western blotting
Recombinant *E. coli* culture was induced with 0.1mM of IPTG, then rGroEL contained bacterial protein extracts was detected western blotting using WEST-one™ Western Blot Detection System (Cat.No.16031).
The rGroEL was detected from total proteins extracted with SMART™ Bacterial Protein Extraction Solution
Lane M, Protein Marker (cat. No. 24051); lane 1, 250 ng of bacterial total protein (BTP); lane 2, 50ng of BTP; lane 3, 10ng of BTP; lane 4, 2ng of BTP

Inclusion Body Isolation

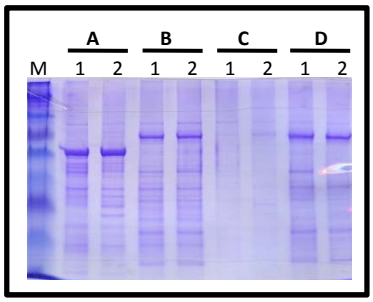

Fig. 4. Isolated inclusion bodies
Each of inclusion body of several expression hosts was isolated and solubilized (followed by Protocol 2) .
Lane A, M15 [rGroEL]; lane B, BL21[rTaq]; lane C, origami [rTaq]; lane D, Rogettagami [rTaq]; lane M, Protein Marker lane 1, SMART™ Bacterial Protein Extraction Solution; lane 2, Competitor's Kit

Better than Sonication

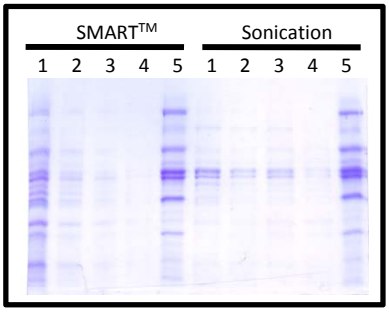

Fig. 5. Each extraction was analyzed by SDS-PAGE
E.coli expressing Taq enzyme was extracted four times using SMART™ Bacterial Protein Extraction Solution or PBS/sonication.
Lane 1, 1 round Extraction; lane 2, 2 round Extraction; lane 3, 3 round Extraction; lane 4, 4 round Extraction; lane 5, Insoluble fraction (pellet)

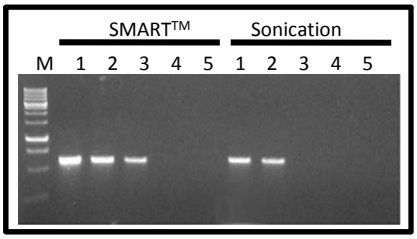

Fig. 6. Comparative study of SMART™ Bacterial Protein Extraction Solution and sonication about protein activity test
In order to estimate protein stability as extraction method, the activity of recombinant Taq DNA polymerase that was 1 round extract of different methods was tested by PCR amplification. (Test for PCR sensitivity after dilution by 1/5 each other.)
Lane M, DNA Marker; lane 1, Total cell protein (TCP) 1μl; lane 2, 1/5 diluted TCP 1μl; lane 3, 1/25 diluted TCP 1μl; lane 4, 1/125 diluted 1μl; lane 5, 1/625 diluted TCP 1μl

RELATED PRODUCT

Product Name	Cat. No.
PRO-PREP™ Protein Extraction Solution (C/T)	17081
SMART™ BCA Protein Assay Kit	21071
PRO-MEASURE™ Protein Measurement Solution	21011
PRO-STAIN™ (I) Prestained Protein Marker	24051
PRO-STAIN™ (II) Prestained Protein Marker	24061
WEST-one™ Western Blot Detection System	16031~16033
WEST-ZOL® plus Western Blot Detection System	16021