DETECTION AND PURIFICATION

CelLytic™ Protein Extraction Reagents

Efficient, yet gentle non-denaturing detergents for extraction of active recombinant proteins

The CelLytic products are a family of protein extraction reagents, specifically formulated to lyse and extract cellular proteins based on the type of expression system. All are meant to rapidly lyse the cells with an easy-to-follow protocol. The CelLytic family is compatible with a wide variety of protease inhibitors, chelating agents, and chaotropes. Because the proteins are in a non-denaturing environment, these reagents do not interfere with standard affinity chromatography. Downstream applications, such as Western blots, gel-shift assays, affinity purification, and reporter detection can be performed without removing the CelLytic reagent. Overall extraction efficiency is consistently higher than other common protocols, such as freeze-thaw or sonication. Although each CelLytic is uniquely formulated, all are flexible enough to be conveniently scaled up to meet any laboratory's needs.

Bacterial Lysis

CelLytic B and CelLytic BII

This is a highly efficient (Fig. 1) yet gentle reagent for the extraction of proteins from bacteria *(E. coli).* This reagent is a proprietary formulation of a non-ionic detergent in 20 mM Trizma®-HCl (pH 7.5). Treatment of bacterial cells with CelLytic B results in rapid extraction of proteins that are suitable for affinity purification and analysis (Fig. 2). CelLytic B is the method of choice for recombinant protein extraction and purification from *E. coli.*

CelLytic BII has double the strength of CelLytic B for small volume extractions. Only 5 ml of the CelLytic BII reagent is required to lyse and extract protein from 1 gram of wet cell paste. This allows CelLytic BII to be used when a higher protein concentration or lower volume is required. The original formula, CelLytic B, requires 10-20 ml of the reagent for 1 gram of wet cell paste.

Features & Benefits

- Scalable for 1 to 25 grams of bacterial cell paste
- No interference with downstream applications such as affinity chromatograpy, IP, and Western blotting
- With the addition of lysozyme, inclusion bodies are ready for resolubilization after two simple washes
- Compatible with protease inhibitors, inhibitor cocktails, chaotropes, salts, chelating agents, and reducing agents

More Efficient Than Conventional Methods

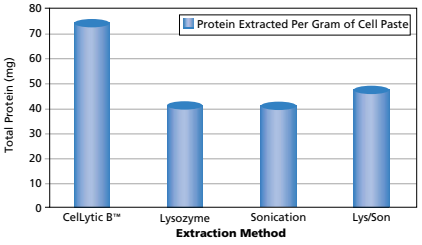
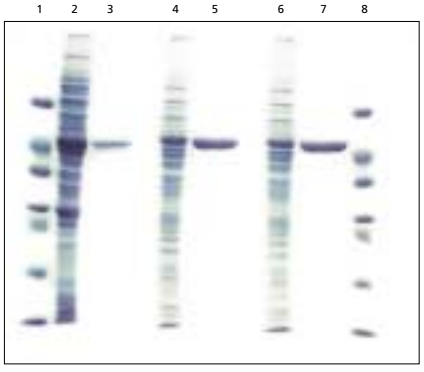


Figure 1. Highest Extraction Efficiency.

One gram of E. coli *cell paste was extracted using CelLytic B, lysozyme, or lysozyme and sonication. The CelLytic B was used at 10 mg/ml of cell paste. The lysozyme treatment was at 10 mg/ml lysozyme (L 7651), 10 mM EDTA (E 5134) for 15 minutes on ice. Sonication time was 2 minutes on ice. Total protein extracted was determined using the Bradford protein assay.*



Compatible for Affinity Chromatography

Figure 2. Affinity Purification.

SDS-PAGE gel of E. coli *cell extracts prepared with CelLytic B used in affinity purification. Lanes 1 and 8: Low molecular weight markers (M 3913). Lanes 2, 4 and 6: Crude cell extracts that contain GST, FLAG and His-tagged fusion proteins. Lanes 3, 5 and 7: GST, FLAG, and His-tagged fusion proteins after affinity purification.*

Product Code	Description	Size
B 3553	CelLytic B Cell Lysis Reagent (standard strength)	50 ml 500 ml
B 3678	CelLytic BII Cell Lysis Reagent (2x strength)	50 ml 500 ml

DETECTION AND PURIFICATION

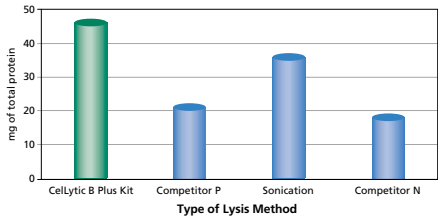


Figure 1. B. subtilis Extraction, 1 gram of cell paste

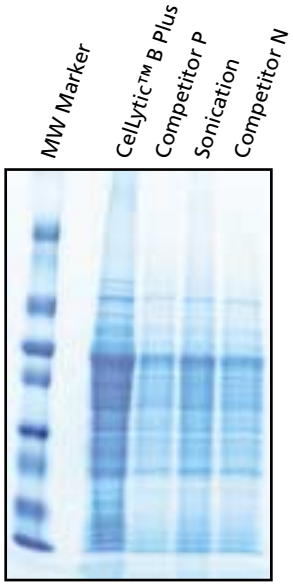


Figure 2. CelLytic B Plus kit vs. Leading Competitors

0.5 grams of Bacillus subtilis were lysed using standard procedures. The lysates were then spun to remove cellular debris and the supernatant was analyzed using Bradford Reagent (Prod. Code B 6916) and 5 µl of lysate was loaded onto a 4-20% Tris-Glycine Polyacylamide Gel to visualize the proteins. The gel was then stained with EZBlue gel staining reagent (Prod. Code G 1041).

CelLytic™ B Plus

For efficient protein extraction of Gram⁺ and Gram⁻ Bacteria

The CelLytic B Plus Kit is designed to efficiently lyse cells and extract proteins from both Gram negative, and difficult to lyse, Gram positive bacteria. This is accomplished using the standard CelLytic B, a proprietary non-ionic detergent in concert with lysozyme, Benzonase®, and protease inhibitors. This complete kit takes the guesswork out of protein extraction from a variety of bacterial species.

Features & Benefits

- Lyse Gram positive and Gram negative bacteria
- More efficient than sonication
- Compatible with affinity purification
- Isolate inclusion bodies for subsequent solubilization
- Non-denaturing cell lysis preserves protein function

Lysis with CelLytic B Plus preserves protein function and is compatible with affinity chromatography. The gentle non-denaturing conditions preserve protein function so that assays can often be performed without removal of lysis reagents. Lysates containing CelLytic B Plus can be applied directly to ANTI-FLAG® M2, and HIS-Select™ Nickel Affinity Gels for direct isolation of tagged recombinant proteins. In addition, insoluble inclusion bodies can be isolated for subsequent solubilization using CelLytic IB and refolding using your method of choice.

Components:	
B 3553	CelLytic B, Bacterial Lysis Reagent
L 3790	Lysozyme Solution
E 1014	Benzonase
P 8849	Protease Inhibitor Cocktail for Histidine-tagged proteins

Product Code	Description	Pkg. Size
CB0050	CelLytic B Plus Kit: Introductory Size (Sufficient for 5 g cell paste)	1 kit
CB0500	CelLytic B Plus Kit (Sufficient for 50 g cell paste)	1 kit

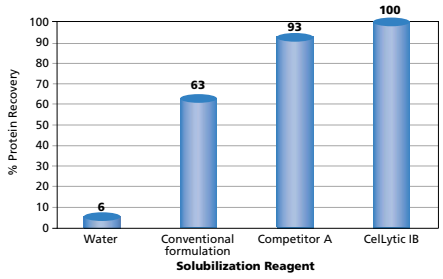


Figure 1. Solubilization of Streptavidin Inclusion Bodies.

Samples of solubilized inclusion body protein were assayed using BCA Reagent (BCA-1). 50 µl of each sample was incubated with BCA reagent at 60 °C for 15 min. The samples were then cooled to room temperature and assayed at 562 nm. The data above has been standardized to the protein recovery of CelLytic IB.

CelLytic™ IB

CelLytic IB was designed to solubilize protein aggregates called inclusion bodies (Fig. 1). In bacteria, inclusion bodies are sometimes formed when recombinant proteins are overexpressed. CelLytic IB was formulated to solubilize the protein of interest for immediate analysis of protein content or refolding procedures.

Product Code	Description	Size
C 5236	CelLytic IB Cell Lysis Reagent	50 ml 500 ml

DETECTION AND PURIFICATION

Mammalian Lysis

CelLytic™ M

CelLytic M is a proprietary detergent solution designed for efficient whole-cell protein extraction from cultured mammalian cells (Fig. 1). It enables efficient and rapid cell lysis and solubilization of proteins for both suspension and adherent cells. Treatment of adherent cells does not require scraping from culture disk. Lysates can be used in many downstream applications without removing the CelLytic M such as reporter gene assays, Western blots/immunoprecipitation, electrophoretic mobility shift assays, phosphatase assays and kinase asssays.

Use 125 µl of CelLytic M for 10^6-10^7 of suspended cells. For adherent cells, use 500-1,000 µL for a 100 mm plate; 200-400 µL for a 35 mm plate.

Product Code	Description	Size
C 2978	CelLytic M Cell Lysis Reagent	50 ml 250 ml

Outperforms other Conventional Methods

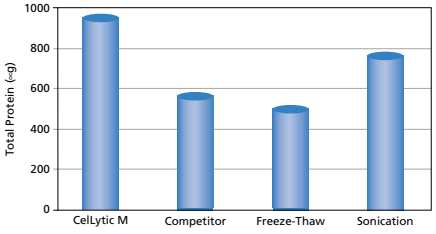


Figure 1. Comparison of Extraction Efficiency.

2 x 10^7 COS cells were washed and divided into equal aliquots, then lysed by one of the methods indicated. Protein amounts were determined by a BCA assay.

CelLytic™ MT

For mammalian tissues, CelLytic MT is an efficient reagent for the extraction of proteins. The lysis buffer consists of a dialyzable mild detergent, bicine, and 150 mM NaCl, resulting in minimal interference with protein interactions and biological activity (Fig 1). CelLytic MT is also used for extraction of cell-line proteins. A volume of 20 ml of CelLytic MT is sufficient for 1 gram of tissue.

It has been tested on the following tissues: rat brain, kidney, muscle, heart, liver, and spleen; mouse brain, kidney and muscle.

Product Code	Description	Size
C 3228	CelLytic MT Cell Lysis Reagent	50 ml 250 ml

Application for Protein DNA Interaction

Figure 1. Gel Shift Assay of Oct-1.

Double Stranded ^{32}P-labeled Oct-1 binding motif oligonucleotide was incubated with CelLytic MT extracts (4 µg total protein). Arrows indicate the Oct-1-DNA complex and free probe.

CelLytic™ Nu-CLEAR™ Extraction Kit

Within this kit is a complete system for preparing nuclear and cytoplasmic protein extracts from mammalian tissue or cultured cell. A number of different procedures in the detailed technical bulletin enable the selection that best fits a particular application. For example, choose between detergent and non-detergent extraction of nuclear protein or between the standard hypotonic lysis buffer for most cell types and isotonic lysis buffer for fragile cells. In addition, the kit provides a procedure for salt reduction from the nuclear extract with dilution buffer. CelLytic Nu-CLEAR offers the flexibility you need for optimal protein extraction. Extracts can be prepared in less than 2 hours and are highly pure since there is little or no cross-contamination between nuclear and cytoplasmic extracts (Fig. 1). Each kit is sufficient for 100 extractions of 100 µl of packed cell volume.

Product Code	Description	Size
N-XTRACT	CelLytic Nu-CLEAR Extraction Kit	1 kit

Highly Purified Nuclear Fractions

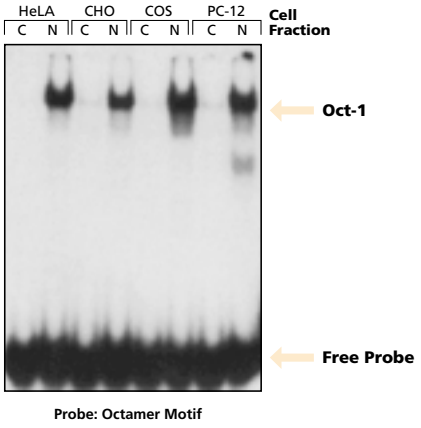


Figure 1. Purity of Cytoplasmic and Nuclear Proteins.

The double stranded ^{32}P-labeled Octamer motif oligonucleotide was incubated with either cytoplasmic fraction (C) or nuclear extract (N) prepared from HeLa, CHO, COS and PC-12 cells using the CelLytic Nu-CLEAR extraction kit.

DETECTION AND PURIFICATION

CelLytic™ MEM Protein Extraction Kit

Components
Lysis and Separation Buffer
Wash Buffer for Cellytic MEM
Protease Inhibition Cocktail for mammalian cells
Sodium Chloride, 4M Solution

The kit offers a fast and convenient method to isolate hydrophobic and raft microdomain associated proteins from cells. The method is based on phase separation and does not require cell membrane isolation. The separated proteins can be used for further experiments such as SDS-PAGE, Western blotting, dot blotting, and immunoprecipitation. The kit has been tested on HeLa, HEK-293, NIH3T3, COS, and CHO cell lines. The kit provides sufficient reagents for 80 tests.

Product Code	Description	Size
CE0050	CelLytic MEM Protein Extraction	1 kit

RIPA Buffer

RIPA (**R**adio-**I**mmuno**p**recipitation **A**ssay) Buffer enables efficient cell lysis and protein solubilization while avoiding protein degradation and interference with the proteins' immunoreactivity and biological activity. RIPA Buffer also results in low background in immunoprecipitation and molecular pull-down assays. Sigma's RIPA Buffer is a ready-to-use 1X solution and is formulated as follows: 150 mM NaCl, 1.0% IGEPAL® CA-630, 0.5% sodium deoxycholate, 0.1% SDS, and 50 mM Tris, pH 8.0. It is also compatible with EZview™ Red Affinity Gels.

Product Code	Description	Size
R 0278	RIPA Buffer	50 ml 500 ml

DETECTION AND PURIFICATION

Plant Lysis

CelLytic P

CelLytic P is a non-ionic detergent-based reagent that offers a convenient method for efficient plant cell lysis and protein solubilization. It is a non-denaturing reagent and maintains protein immunoreactivity and biological activity (Fig. 1). CelLytic P is efficient, rapid, and ready to use. It contains bicine buffer, which is preferable for many biological activities. Use of CelLytic P enables extraction of proteins from less than one gram to hundreds of grams of fresh or frozen leaves, employing the same short procedure. It has been tested on leaves from four plant models: tobacco, tomato, spinach, and *arabidopsis*.

Product Code	Description	Size
C 2360	CelLytic P Cell Lysis Reagent	50 ml 250 ml

Detection of DNA/Protein Interactions

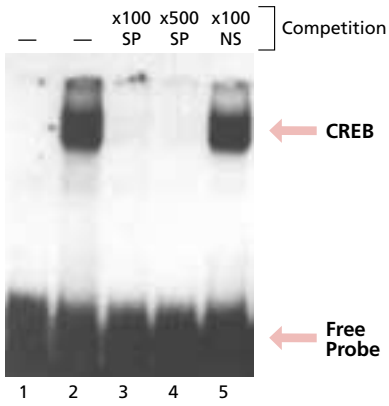


Figure 1. Compatibility with Gel Shift Assay.

Protein extracts were prepared with the CelLytic P reagent from spinach leaves. A double stranded ^{32}P labeled CREB oligonucleotide probe was incubated with 28 µg of the whole cell extract (lanes 2-5) or without whole cell extract (lane 1, free probe). Binding reactions with the extracts were performed in the absence [–] of competitor oligonucleotide (lanes 1-2) or in the presence of 100 or 500 fold excess of unlabeled CREB binding motif oligonucleotide (specific competitor [SP], lane 3 and lane 4, respectively) or in the presence of 100 fold excess of unlabeled oligonucleotide (non specific competitor, [NS] lane 5). Binding reactions were run on a non-denaturing 6% polyacrylamide gel, dried, and imaged on X-ray film. The arrows indicate the CREB-DNA complex and the free probe.

CelLytic™ PN Extraction Kit

This kit is for the rapid isolation of nuclei and extraction of functional nuclear proteins from plant leaves (Fig. 1). Nuclei or nuclear proteins can be extracted from a few grams to hundreds of grams of fresh or frozen leaves. The nuclear protein extract is suitable for the detection of DNA-protein interactions using gel-shift assay, DNase-I footprinting analysis, as well as Western blot assay and similar techniques. The isolated nuclei can also be used as a source for chromatin, genomic DNA, RNA, etc. The kit provides a detailed protocol for nuclei isolation and protein extraction from four plant models: tobacco, tomato, spinach, and *arabidopsis*.

Product Code	Description	Size
CelLyt-PN-1	CelLytic P Cell Lysis Kit	1 kit

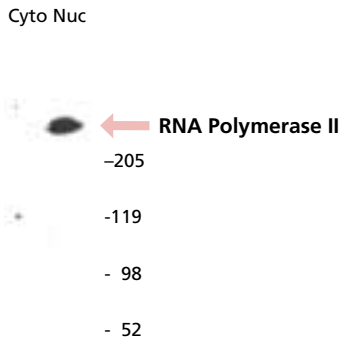


Figure 1. Detection of RNA polymerase II in Tomato Nuclear versus Cytoplasmic extracts, prepared with CelLytic PN Kit.

The Extracts were run on SDS-PAGE and blot-hybridized to anti-RNA Polymerase II antibody.

Yeast Lysis

CelLytic™ Y

CelLytic Y Cell Lysis Reagent is versatile, phosphate-free, and non-denaturing. It provides an effective method for cell lysis and protein solubilization (Fig. 1). Target proteins maintain immunoreactivity or biological function. The protocol is brief and performed at room temperature. No extreme conditions or glass beads are required. Use only 2.5-5 ml/gram of yeast cells. Supplement with DTT (5 mM) for enhanced protein yield.

Product Code	Description	Size
C 4482	CelLytic Y Cell Lysis Reagent	50 ml 500 ml

More Efficient Than Glass Beads

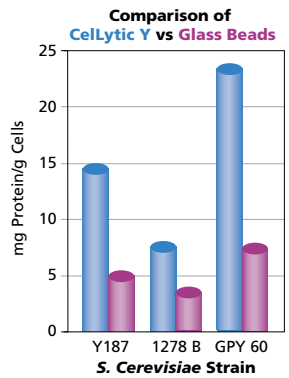


Figure 1. CelLytic Y vs. Glass Beads Method.

One gram of S. cerevisiea washed cell pellet was extracted with the following: CelLytic Y, or glass beads procedure. Protein was determined using BCA protein assay.