

Cell Lysis and Recombinant Protein Extraction

CellLytics

CellLytic™ P Plant Cell Lysis Reagent

C 2360 This is a non-ionic detergent-based reagent, which offers a convenient method for efficient plant cell lysis and protein solubilization. It's a non-denaturing reagent and maintains protein immunoreactivity and biological activity. CellLytic P is efficient, rapid, and ready to use. It contains bicine buffer, which is preferable for many biological activities. Use of CellLytic 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 (but not limited to) four plant models: tobacco, tomato, spinach, and *Arabidopsis*.
S: 23-24/25

Detection of DNA/Protein Interactions

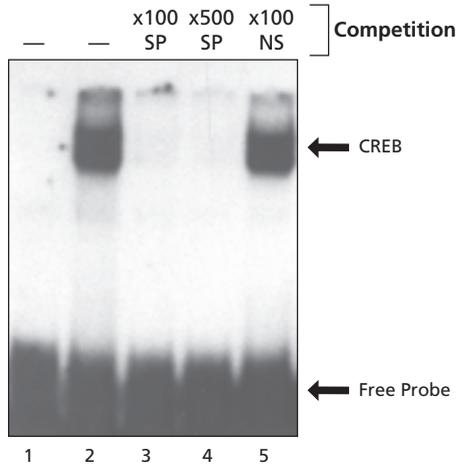


Figure 1. Compatibility with Gel Shift Assay. Protein extracts were prepared with the CellLytic-P reagent from spinach leaves. A double stranded ³²P labeled CREB oligonucleotide probe was incubated with 28 μg of the whole cell extract (lanes 2-5) or without whole cell extract (lane 1, free probe). Binding reactions with the extracts were performed in the absence [-] of competitor oligonucleotide (lanes 1-2) or in the presence of x100 or x500 fold excess of unlabeled CREB binding motif oligonucleotide (specific competitor [SP], lane 3 and lane 4, respectively) or in the presence of x100 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.

CellLytic™ PN Extraction Kit

CELLYT-PN-1 For plant leaves 1 kit
 2-8°C
 WET ICE
 This kit is for the rapid isolation of nuclei and extraction of functional nuclear proteins from plant leaves. Nuclei or nuclear proteins can be extracted from a few grams to hundreds of grams of fresh and 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*.
 1 kit sufficient for 30 extractions 20 g fresh or frozen leaves

- Components:**
 Nuclei Isolation Buffer 4x (NIB), Percoll, Sucrose 2.3 M, TRITON® X-100 10% solution, Extraction Buffer, Nuclei PURE Storage Buffer, Filter Mesh 100,
 R: 20/22-37/38-41 S: 26-36

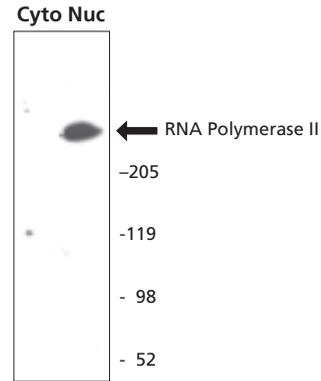


Figure 1. Detection of RNA polymerase II in Tomato Nuclear versus Cytoplasmic extracts, prepared with CellLytic-PN Kit. The Extracts were run on SDS-PAGE and blot-hybridized to anti-RNA Polymerase II antibody.

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Compatibility with Gel Shift Assay

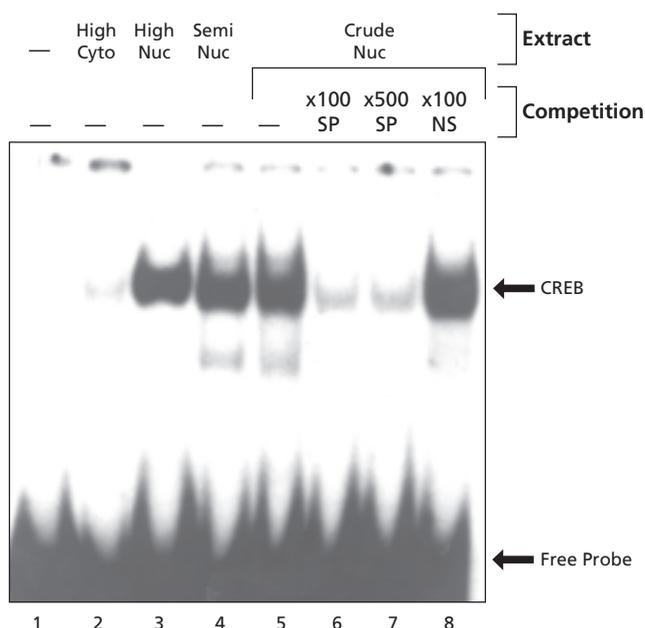


Figure 2: Gel Shift analysis of protein-DNA complex formed between CREB DNA binding site and CREB protein in Tobacco Nuclear extracts.

Protein extracts were prepared with the CellLytic-PN kit from nuclei isolated at three levels of purity (high, semi-pure, crude). A double stranded ³²P labeled CREB oligonucleotide probe was incubated with 1 µg of cytoplasmic or nuclear extract. Binding reactions with the nuclear extracts were performed in the absence [-] of competitor oligonucleotide (lanes 1-5) or in the presence of x100 or x500 fold excess of unlabeled CREB binding motif oligonucleotide (specific competitor [SP], lane 6 and lane 7, respectively) or in the presence of x100 fold excess of unlabeled oligonucleotide (non specific competitor, [NS] lane 8).

Lane 1: Free probe without extract.

Lane 2: Cytoplasmic fraction present in the supernatant of "High" level of purity.

Lane 3: Nuclear proteins isolated by "High" level of purity.

Lane 4: Nuclear proteins isolated by "Semi-pure" level of purity.

Lanes 5-8: Nuclear proteins isolated by "Crude" level of purity.

Binding reactions were run on a non-denaturing 6% polyacrylamide gel, dried and imaged on film. The arrows indicate the CREB-DNA complex and the free probe.

CellLytic™ B Bacterial Cell Lysis Reagent

B 3553 Standard strength 50 mL

RT A gentle, highly efficient reagent for the extraction of proteins from bacteria (*E. coli*). A proprietary formulation of a non-ionic detergent in 20 mM Trizma®-HCl (pH 7.5). Treatment of bacterial cells with CellLytic B results in rapid extraction of proteins that are suitable for affinity purification and analysis.

Features and Benefits

- Scalable for 1 to 25 grams of bacterial cell paste
- No interference with downstream applications such as affinity chromatography, IP, and Western blotting
- Compatible with protease inhibitors, inhibitor cocktails, chaotropes, salts, chelating agents and reducing agents

CellLytic™ B-II Bacterial Cell Lysis Reagent

B 3678 2× strength 50 mL

RT A gentle, highly efficient reagent for the extraction of proteins from bacteria (*E. coli*). A proprietary formulation of a non-ionic detergent in 20 mM Trizma®-HCl (pH 7.5). Treatment of bacterial cells with CellLytic B results in rapid extraction of proteins that are suitable for affinity purification and analysis.

Features and Benefits

- Scalable for 1 to 25 grams of bacterial cell paste
- No interference with downstream applications such as affinity chromatography, IP, and Western blotting
- Compatible with protease inhibitors, inhibitor cocktails, chaotropes, salts, chelating agents and reducing agents

CellLytic™ B Plus Kit

NEW The CellLytic™ B Plus Kit contains all of the reagents and chemicals necessary to lyse both Gram negative and Gram positive bacteria. This kit also includes protease inhibitors to help prevent the proteolytic breakdown of proteins. This kit is compatible with FLAG®, HIS-Select™, and glutathione S-transferase based affinity chromatography protein purification systems. The detergent included in the CellLytic B lysis Reagent can be removed from the protein by dialysis or ammonium sulfate precipitation, if necessary. The final purity of the recombinant product is usually higher than that obtained from traditional extraction methods. This is because the crude protein solution containing the non-ionic detergent eliminates much of the non-specific binding that occurs during chromatography. The mild detergent does not interfere with many enzyme assays or protein assays.

Components:

CellLytic™ B Bacterial Cell Lysis Reagent

Lysozyme from chicken egg white

Benzonase

Protease Inhibitor Cocktail for use in purification of (histidine)-tagged proteins

CB0050 CellLytic B Plus Kit Introductory Size 1 kit

-20-0°C For bacterial lysis

WET ICE This introductory size kit can lyse up to 5 grams of fresh or frozen cell paste.

CB0500 CellLytic B Plus Kit 1 kit

-20-0°C For bacterial lysis

WET ICE Fifty grams of fresh or frozen cell paste can be lysed with this kit.

CellLytic™ IB Inclusion Body Solubilization Reagent

C 5236 CellLytic IB was designed to solubilize 25 mL

RT protein aggregates called inclusion bodies, 100 mL

NEW which are formed in bacteria when recombinant proteins are overexpressed. CellLytic IB was formulated to solubilize the protein of interest for immediate analysis of protein content or refolding procedures.
R: 22-36/38 S: 26-36

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(Continuation of)

CellLytic™ IB Inclusion Body Solubilization Reagent

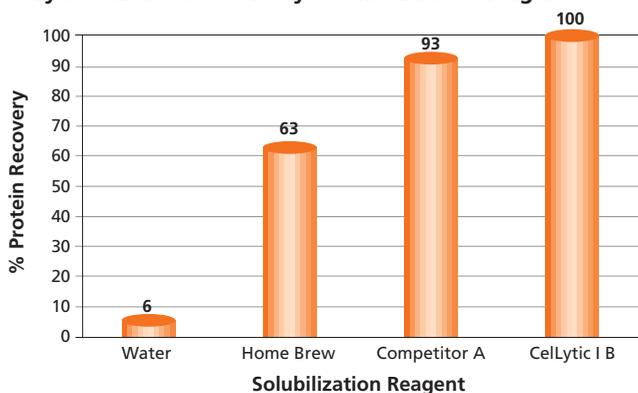


Figure 1. Solubilization of Streptavidin Inclusion Bodies.

Samples of solubilized inclusion body protein were assayed using BCA Reagent (BCA-1). 50 µl of each sample were 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 CellLytic IB.

Protease Inhibitors

Protease Inhibitor Cocktail for plant cell and tissue extracts

P 9599 DMSO solution 1 mL
 [-20°C] A mixture of protease inhibitors with broad specificity for the inhibition of serine, cysteine, aspartic, and metalloproteases, and aminopeptidases. Contains 4-(2-aminoethyl)benzenesulfonyl fluoride (AEBSF), bestatin, pepstatinA, E-64, leupeptin, and 1,10-phenanthroline. One ml is recommended for the inhibition of proteases extracted from 30 g of plant tissue in a total volume of 100 ml. Tested for inhibition in extracts from *Phaseolus vulgaris* (kidney bean), *Pisum sativum* (pea), *Triticum aestivum* (wheat), *Nicotiana tobaccum* (tobacco), and *Arabidopsis thaliana* (arabidopsis).

Protease Inhibitor Cocktail for use with bacterial cell extracts

P 8465 Lyophilized powder 5 mL
 [-20°C] A mixture of protease inhibitors with broad specificity for the inhibition of serine, cysteine, aspartic and metallo-proteases, and aminopeptidases. Contains 4-(2-aminoethyl)benzenesulfonyl fluoride (AEBSF), pepstatin A, E-64, bestatin, and sodium EDTA. Five ml is recommended for the inhibition of proteases extracted from 20 g of *Escherichia coli*. Supplied with a vial of DMSO.

Protease Inhibitor Cocktail for General Use

P 2714 Lyophilized powder 1 each
 [-0°C] A mixture of water-soluble protease inhibitors with broad specificity for the inhibition of serine, cysteine, aspartic, and metallo-proteases. Contains 4-(2-aminoethyl)benzenesulfonyl fluoride (AEBSF), E-64, bestatin, leupeptin, aprotinin, and sodium EDTA. One bottle makes 100 ml of cocktail. One ml is recommended for the inhibition of proteases equivalent to 1 mg of USP pancreatin.

Protein Extraction for Proteomics

Chloroplast Isolation Kit

CP-ISO The chloroplast isolation kit provides an efficient procedure for isolating in-tact chloroplasts from plant leaves. In-tact chloroplasts serve as the best starting material for studies of chloroplast processes such as carbon assimilation, electron flow and phosphorylation, metabolic transport, and protein targeting.

Kit Components

Name	Amount
Chloroplast Isolation Buffer 5x (CIB)	500 ml
Percoll	100 ml
Bovine Serum Albumin (BSA)	3 g
Filter Mesh 100	4 each

