For life science research only. Not for use in diagnostic procedures. FOR *IN VITRO* USE ONLY.

cOmplete Lysis-B (2×), EDTA-free

Reagent set for highly efficient protein extraction from bacteria and insect cells by rapid lysis and concurrent protection of extracted proteins against a multitude of proteases. Suitable for downstream purification using IMAC

Cat. No. 04 719 948 001

Version October 2005

Store cOmplete tablets at +2 to +8°C
Store Lysis-B Reagent at +15 to +25°C

1. What this Product Does

Number of Reactions

The set is designed for

- the lysis of up to 5,000 ml of bacterial culture with an OD_{600} of 1.5 – 3.0 (corresponding to approx. 20 g of wet bacterial cell paste)

OR

- the lysis of up to 20 g of wet insect cell paste (or up to 400 plates of insect cell culture grown in monolayer [100 mm])

Kit Contents

Label	Contents
Lysis-B Reagent (2× conc.)	100 ml
cOmplete, Mini, EDTA-free Protease Inhibitor Cocktail Tablets	• 20 tablets, supplied in *EASYpacks* (foil blisters) • Each tablet is sufficient for a volume of 5 ml solution.

Storage and Stability

- If stored at room temperature the Lysis-B Reagent is stable through the expiration date printed on the label.
- If stored dry at +2 to +8°C the cOmplete, Mini Protease Inhibitor Cocktail Tablets are stable through the expiration date printed on the label.

Additional Equipment and Reagents Required

For inclusion body purification:

- Lysozyme*
- Sterile water to prepare a 1:20 dilution of the Lysis-B Reagent (for washing inclusion bodies).

** available from Roche Applied Science*

Application

cOmplete Lysis-B (2×) EDTA-free is intended for the rapid cell lysis of bacteria cells in only 10 minutes with simultaneous inhibition of protease activity in the cell lysate.

Proteases are released during the extraction of proteins from bacteria, resulting in rapid degradation of proteins (1). cOmplete Lysis-B (2×) EDTA-free enables highly efficient protein extraction from several common bacterial host strains (especially BL21 strains) and the simultaneous inhibition of a multitude of proteases, including serine proteases and cysteine proteases.

Lysis-B Reagent purifies soluble proteins and inclusion bodies to near homogeneous levels. The reagent has also been tested for the extraction of proteins from insect cells infected by baculovirus (a sample protocol is provided).

Q cOmplete, Mini, EDTA-free tablets are employed to stabilize those extracts where the stability or activity of metal-containing proteins must not be affected. Since EDTA interferes with IMAC (immobilized metal affinity chromatography), cOmplete, Mini, EDTA-free is preferentially used in the isolation process of Poly-His tagged fusion proteins or subsequent assays. cOmplete, Mini, EDTA-free tablets efficiently inhibit a wide range of serine and cysteine proteases, but not metalloproteases.

2. How To Use this Product

2.1 Before You Begin

General Remarks

The expression of recombinant proteins in bacteria often results in the formation of inclusion bodies containing incorrectly folded, and therefore mainly insoluble, proteins.

Lysis-B Reagent effectively extracts both soluble and insoluble (inclusion body) proteins. Before performing a large-scale extraction of the proteins, extraction on a small scale is recommended in order to analyze the solubility of the recombinant proteins.

The addition of lysozyme to digest the cell debris and improve the purity of inclusion body proteins is strongly recommended for the purification of inclusion bodies. Lysozyme is eliminated during subsequent washing steps.

Safety precautions

Observe the usual precautions to be taken when handling chemicals.

⚠ Consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation of Working Solutions

- One cOmplete, Mini, EDTA-free tablet is sufficient for the inhibition of the proteolytic activity in 5 ml Lysis-B Reagent. If very high proteolytic activity is present, one tablet should be used for 3.5 ml Lysis-B Reagent. Reverse Lysis-B Reagent two times to ensure complete mixing. Dissolve the tablet in 5 ml of the provided Lysis-B Reagent by incubating for 2 min at RT, afterwards vortex shortly.
- For inclusion body purification: Dissolve the lysozyme in Lysis-B Reagent containing cOmplete to a final concentration of 10 mg/ml. Use a fresh lysozyme solution each time.

⚠ The addition of DNase I to the extraction reagent (f.c. 50 – 100 U/ml) can help eliminate the viscosity of the extract by removing nucleic acids.

2.2 Protocol for Small-Scale Protein Extraction

(1.5 ml bacterial culture, OD_{600} 1.5 – 3.0)

1 Harvest bacterial cells by centrifugation at 5,000 rpm for 10 min.
Q Either fresh cells or cells frozen at −70°C can be used.

2 • Remove the supernatant and resuspend the cells in 150 µl of Lysis-B Reagent containing cOmplete, EDTA-free by either pipetting up and down the mixture or vortexing until the cell suspension is homogeneous.
• Vortex 1 min.

3 Centrifuge at 13,000 rpm for 5 min to pellet any insoluble proteins and cell debris.

4 • Carefully remove the supernatant containing the soluble protein fraction.
• To analyze the insoluble protein fraction, resuspend the pellet in 150 µl of Lysis-B Reagent containing c*O*mplete, EDTA-free (repeat Step 2).
• Use 10 µl each of the soluble and insoluble fraction for SDS-PAGE or Western blotting assay to determine the solubility of the recombinant protein.

⚠ If purification of inclusion bodies is required, proceed to Step 5.

5 • To purify inclusion bodies, add lysozyme (f.c. 400 µg/ml; use a 10 mg/ml stock solution) to the resuspended pellet (insoluble fraction generated in Step 4), and vortex for 1 min.
• Add 1 ml of 1:20 diluted Lysis-B Reagent containing c*O*mplete, EDTA-free to the suspension and vortex for 1 min.

6 • Centrifuge inclusion bodies at 13,000 rpm for 10 min.
• Resuspend the pellet in 1 ml of 1:20 diluted Lysis-B Reagent containing c*O*mplete and vortex for 1 min.

7 Perform Step 6 two more times.

8 • Resuspend the final inclusion body pellet in 300 µl of sterile water or desired buffer.
• Analyze 10 - 20 µl of the sample by SDS-PAGE assay.

2.3 Protocol for Medium-Scale Bacterial Protein Extraction (40 ml bacterial culture, $OD_{600\ nm}$ 1.5 – 3.0)

1 Harvest bacterial cells by centrifugation at approx. 3,000 × *g* (*e.g.,* 5,000 rpm for Beckman JA20 rotor) for 10 minutes.

Ⓠ Either fresh cells or cells frozen at −70°C can be used.

2 • Remove the supernatant and resuspend the cells in 2.5 ml of Lysis-B Reagent containing c*O*mplete, EDTA-free by either pipetting up and down the mixture or vortexing until the cell suspension is homogeneous.
• Shake the mixture gently for another 10 – 20 min at room temperature (RT).

3 Centrifuge at 27,200 × *g* (*e.g.,* 15,000 rpm for Beckman JA20 rotor) for 15 min to pellet any insoluble proteins and cell debris.

⚠ Expect to recover more than 90% of the soluble proteins from the first extraction. An additional extraction is not usually required, but it might help increase the yield of soluble proteins.

Ⓠ If purification of inclusion bodies is required, proceed to Step 4.

4 To purify inclusion bodies, add 2.5 ml Lysis-B Reagent containing c*O*mplete, EDTA-free to the pellet (insoluble pellet fraction generated in Step 3) and resuspend by vortexing or pipetting.

5 • Add lysozyme (f.c. 400 µg/ml; use a 10 mg/ml stock solution) to the mixture.
• Mix well and incubate at RT for 5 min.
• Add 15 ml of 1:20 diluted Lysis-B Reagent containing c*O*mplete, EDTA-free to the suspension.
• Vortex briefly.

6 • Centrifuge inclusion bodies at 27,200 × *g* for 15 min.
• Resuspend the pellet in 20 ml of 1:20 diluted Lysis-B Reagent containing c*O*mplete, EDTA-free .
• Vortex briefly.

7 Perform Step 6 two more times.

8 • Resuspend the final inclusion body pellet in denaturing agents.
• Proceed further with refolding or purification procedures.

2.4 Protocol for Large-Scale Bacterial Protein Extraction (250 ml bacterial culture, OD_{600} 1.5-3.0)

⚠ Increase the volume of reagent accordingly for larger volumes of bacterial cultures.

1 Harvest bacterial cells by centrifugation at 3,440 × *g* (*e.g.,* 5,000 rpm for Beckman JA17 rotor) for 10 min.

Ⓠ The cells can either be used fresh or frozen at −70°C.

2 • Remove the supernatant and resuspend the cells in 5 - 10 ml of Lysis-B Reagent containing c*O*mplete, EDTA-free by either pipetting up and down the mixture or vortexing until the cell suspension is homogeneous.
• Shake the mixture gently for another 10 min at room temperature (RT).

3 Centrifuge at 27,000 × *g* (*e.g.,* 14,000 rpm for Beckman JA17 rotor) for 15 min to pellet any insoluble proteins and cell debris.

⚠ Expect to recover more than 90% of the soluble proteins from the first extraction. An additional extraction is not usually required, but it might help increase the yield of soluble proteins.

Ⓠ If purification of inclusion bodies is required, proceed to Step 4.

4 For inclusion body purification, add 5 – 10 ml of Lysis-B Reagent containing c*O*mplete, EDTA-free to resuspend the pellet (insoluble fraction generated in Step 3) and resuspend by vortexing or pipetting.

5 • Add lysozyme (f.c. 200 µg/ml; use a 10 mg/ml stock solution) to the mixture.
• Mix well and incubate at RT for 5 minutes.
• Add 100 ml of 1:20 diluted Lysis-B Reagent containing c*O*mplete, EDTA-free to the suspension.
• Mix by vortexing

6 • Centrifuge inclusion bodies at 27,000 × *g* for 15 min.
• Resuspend the pellet in 100 ml of 1:20 diluted Lysis-B Reagent containing c*O*mplete, EDTA-free .
• Vortex briefly.

7 Perform Step 6 two more times.

⚠ After the final centrifugation, proceed to Step 8 without resuspension.

8 • Resuspend the final inclusion body pellet in denaturing agents.
• Proceed further with refolding or purification procedures.

2.5 Protocol for Protein Extraction from Insect Cells – Sample Method I (Monolayer Culture)

1 • Remove (decant) culture medium from the adherent cells grown in a 100 mm plate.

Optional: Wash cells once in washing buffer (*e.g.,* PBS*).
• Add 0.25 - 0.5 ml of Lysis-B Reagent containing c*O*mplete, EDTA-free .

2 • Briefly incubate the plate on a shaker.
• Collect the lysate by scraping.
• Transfer lysate to a centrifuge tube.

3 Centrifuge the lysed cells at 27,000 × *g* for 15 min. The soluble proteins are separated from the insoluble fraction during centrifugation.

4 Remove the supernatant containing soluble protein and proceed with further analysis.

2.6 Protocol for Protein Extraction from Insect Cells – Sample Method II (Suspension Culture

1 • Collect cells by low speed centrifugation (*e.g.*, 450 × *g*) for 5 min.
• Decant the supernatant.
• Wash cells once with washing buffer (*e.g.,* PBS) and centrifuge for 5 min at low speed by using a weighted centrifuge tube.
• Remove the supernatant.

2 • Determine the wet weight of the cell pellet.
• Add 5 ml of Lysis-B Reagent containing c*O*mplete, EDTA-free for each g of wet cell pellet.

3 Resuspend pellet and shake the suspension for 10 min.

4 Centrifuge the lysed cells at 27,000 × *g* for 15 min. The soluble proteins are separated from the insoluble fraction during centrifugation.

5 Remove the supernatant containing soluble protein and proceed with further analysis.

3. Typical Result

1.5 ml Bl21 DE3 pLysS cells expressing green fluorescent protein (GFP) were harvested by centrifugation at an OD_{600} of 1.5 - 2 and resuspended in 0.2 ml of Lysis-B Reagent in the presence of c*O*mplete. The extracted proteins were analyzed by SDS-PAGE (5 µl/lane).
M: marker
W: whole fraction
S: supernatant fraction
P: pellet fraction

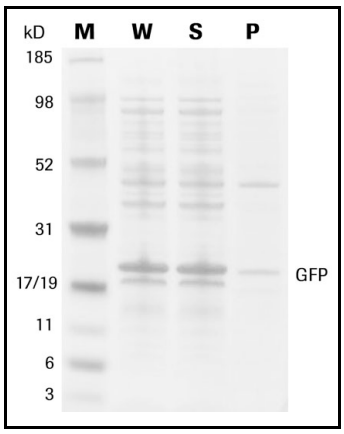

Fig. 1: SDS-PAGE analysis and Coomassie blue staining of proteins extracted from *E. coli* BL21 DE3 cells overexpressing GFP.

c*O*mplete, Mini, EDTA-free Protease Inhibitor Cocktail Tablets were dissolved in Lysis-B Reagent and maintained full functionality for inhibition of a multitude of proteases. Typical values for the inhibition of different proteases and protease mixtures by c*O*mplete, Mini in Lysis-B Reagent are shown in table 1.

Table 1: Inhibition of different proteases by c*O*mplete, EDTA-free Protease Inhibitor Tablets.

Protease or protease mixture	Enzyme concentration (µg/ml)	% inhibition after immediate addition to the protease
Pancreatic extract	20	92%
Chymotrypsin	2.0	92%
Trypsin	0.2	96%
Papain	20	100%

One c*O*mplete, Mini, EDTA-free tablet was added per 5 ml Lysis-B Reagent. Proteolytic activity was determined with the Roche Applied Science Universal Protease Substrate (casein, resorufin-labeled*). When extractions or single-step isolations are necessary in the acid pH range, simply include pepstatin* along with c*O*mplete, Mini, EDTA-free tablets to ensure aspartic (acid) protease inhibition. All experiments were performed at room temperature.

4. Troubleshooting

Observation	Possible Cause	Recommendation
Low protein yield	Insufficient lysis of bacterial cells	Freeze the cells prior to extraction. This helps increase the cellular breakage.
		The addition of lysozyme can help break the cells more efficiently (f.c. 200 - 500 µg/ml).
		Increase the amount of Lysis-B Reagent containing c*O*mplete per gram of wet cell paste (up to 8 ml/g wet cell paste).
	Insufficient lysis of your particular bacterial strain	Freeze/thaw bacterial cells prior to extraction.
		The addition of lysozyme can help break the cells more efficiently (f.c. 200 - 500 µg/ml).
	Insoluble protein	Check the pellet fraction to analyze whether the protein of interest is located in inclusion bodies.
Viscosity of extract too high	Presence of DNA	Add DNase I to remove nucleic acids from the extract (f.c. 50 – 100 U/ml).

5. Additional Information on this Product

Product Description

The c*O*mplete Lysis-B (2×), EDTA-free bacterial protein extraction reagent (Lysis-B Reagent) contains a mild, double-concentrated, non-ionic detergent in 20 mM Tris/HCl (pH 7.5). This reagent allows very efficient and gentle extraction of proteins, especially recombinant proteins, from bacteria in small volumes. This simple extraction method completely eliminates the need for mechanical disruption (*e.g.*, standard sonication). Rapid cell lysis occurs in just 10 minutes at room temperature. The protein yields obtained with this kit are significantly higher compared to those obtained by using sonication.

Lysis-B Reagent is used to extract soluble proteins as well as inclusion bodies from whole bacterial lysates. The reagent extracts proteins from fresh and frozen cells. The protocols have been tested with several different bacterial strains and are especially suitable for *E. coli* BL21 cells. As Lysis-B Reagent is based on a Tris buffer system, Tris-HCl buffers are recommended for subsequent protein purification.

Lysis-B Reagent has also been used successfully to extract proteins from insect cells infected with baculovirus.

Proteases are ubiquitous in all living cells. As soon as cells are disrupted, proteases are released and can quickly degrade any protein. This can drastically reduce the yield of protein during isolation and purification. The c*O*mplete, Mini, EDTA-free tablets, provided with this kit, allow the inhibition of a broad spectrum of proteases but not metalloproteases. In contrast to other c*O*mplete tablets they do not contain EDTA, thus leaving the stability and the function of metal-dependent proteins unaffected. The affinity purification of Poly-His tagged fusion proteins via IMAC (immobilized metal affinity chromatography) is also facilitated (no dialysis necessary).

Due to the optimized composition of the tablets they show excellent inhibition of serine and cysteine proteases and are therefore very well suited for the protection of proteins isolated from bacteria. c*O*mplete, Mini, EDTA-free contains both irreversible and reversible protease inhibitors. Metalloproteases and aspartic proteases are not inhibited. c*O*mplete, Mini, EDTA-free tablets eliminate the time-consuming search for the right protease inhibitor. The ready-to-use water-soluble, non-toxic tablets work optimally with the kit´s Lysis Reagent

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References

1 North, M.J. (1969) in: Proteolytic Enzymes - A Practical Approach (Beynon, P.J. & Bond, J.S. eds.), IRL press Oxford, pp. 117-119.

Quality Control

The inhibitory power of c*O*mplete, Mini, EDTA-free has been demonstrated with many proteases and protease mixtures. In these experiments substantially higher concentrations of proteases were used compared to the concentration usually present in extracts. The inhibitory activity of each lot is tested with a concentrated pancreas extract and a concentrated pronase solution. The proteolytic activities are thereby typically inhibited by 94% after one hour (detection with Universal Protease Substrate, casein, resorufin-labeled*).

The efficiency of cell lysis using Lysis-B Reagent is determined for each lot by functional testing.

6. Supplementary Information

6.1 Text Conventions

To make information consistent and memorable, the following text conventions are used in this package insert:

Text Convention	Use
Numbered Instructions labeled ❶, ❷,etc.	Steps in a procedure that must be performed in the order listed
Asterisk *	Denotes a product available from Roche Applied Science

Symbols

In this package insert the following symbols are used to highlight important information:

Symbol	Description
	Information Note: Additional information about the current topic or procedure.
⚠	Important Note: Information critical to the success of the procedure or use of the product.

Abbreviations

In this Instruction Manual the following abbreviations are used:

Abbreviation	Meaning
f.c.	final concentration
PAGE	polyacrylamide gel electrophoresis
RT	room temperature

6.2 Ordering Information

Roche Applied Science offers a large selection of reagents and systems for life science research. For a complete overview of related products and manuals, please visit and bookmark our home page www.roche-applied-science.com.
For additional information on protease inhibition, please visit or Special Interest Site at: www.roche-applied-science.com/proteaseinhibitor

	Product	Pack Size	Cat. No.
Complete Lysis	Lysozyme	10 g	10 837 059 001
	TriPure Isolation Reagent	50 ml 200 ml	11 667 157 001 11 667 165 001
	DNase I from bovine pancreas	100 ml sterile	11 284 932 001
	DNase I recombinant	2 × 10,000 U	04 536 282 001
cOmplete Protease Inhibitor Cocktail Tablets in EASYpacks	cOmplete	20 tablets in foil blisters (for 50 ml each)	04 693 116 001
	cOmplete, Mini	30 tablets in foil blisters (for 10 ml each)	04 693 124 001
	cOmplete, EDTA-free	20 tablets in foil blisters (for 50 ml each)	04 693 132 001
	cOmplete, Mini, EDTA- free	30 tablets in foil blisters (for 10 ml each)	04 693 159 001
cOmplete Protease Inhibitor Cocktail Tablets in glass vials	cOmplete	20 tablets in a glass vial (for 50 ml each) 3 × 20 tablets in a glass vial (for 50 ml each)	11 697 498 001 11 836 145 001
	cOmplete, Mini	25 tablets in a glass vial (for 10 ml each)	11 836 153 001
	cOmplete, EDTA-free	20 tablets in a glass vial (for 50 ml each)	11 873 580 001
	cOmplete, Mini, EDTA- free	25 tablets in a glass vial (for 10 ml each)	11 836 170 001
Kits and Sets	Pefabloc SC PLUS	Set I: contains 100 mg Pefabloc SC and 5 ml PSC protector solution Set II: contains 1g Pefabloc SC and 2 × 25 ml PSC protector solution	11 873 601 001 11 873 628 001
	Protease Inhibitor Set	Small quantities of 10 most commonly used protease inhibitors	11 206 893 001
	Universal Protease Substrate (Casein, resorufin-labeled)	15 mg 40 mg	11 080 733 001 11 734 334 001
Individual Protease Inhibitors	Aprotinin	10 mg 50 mg 100 mg	10 236 624 001 10 981 532 001 11 583 794 001
	Bestatin	10 mg 50 mg	10 874 515 001 11 359 070 001
	Calpain Inhibitor I	25 mg	11 086 090 001
	Calpain Inhibitor II	25 mg	11 086 103 001
	Chymostatin	10 mg	11 004 638 001
	E-64	5 mg 10 mg 25 mg	11 585 673 001 10 874 523 001 11 585 681 001
	Leupeptin	5 mg 25 mg 50 mg 100 mg	11 017 101 001 11 017 128 001 11 034 626 001 11 529 048 001
	α_2-Macroglobulin	25 inhibitory units	10 602 442 001
	Pefabloc SC	100 mg 500 mg 1 g	11 429 868 001 11 585 916 001 11 429 876 001
	Pepstatin	2 mg 10 mg 50 mg	10 253 286 001 11 359 053 001 11 524 488 001
	PMSF	1 g 10 g 25 g	10 236 608 001 10 837 091 001 11 359 061 001
	TLCK – HCl	100 mg 250 mg	10 874 485 001 10 874 493 001
	Trypsin Inhibitor (chicken, egg white)	1 g	10 109 878 001
	Trypsin Inhibitor (soybean)	50 mg 500 mg	10 109 886 001 10 109 894 001
Buffers	Buffers in a Box, Premixed PBS Buffer, 10×	4 l	11 666 789 001

6.3 Notice to Purchaser

Disclaimer of License

Bacterial protein extraction reagent technology is protected by U.S Patent 6,174,704.

Trademarks

COMPLETE, BUFFERS IN A BOX, and TRIPURE are Trademarks of Roche.

PEFABLOC is a trademark of Pentapharm AG, Basel, Switzerland.

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To call, write, fax, or email us, visit the Roche Applied Science home page, www.roche-applied-science.com, and select your home country.

Country-specific contact information will be displayed.

Use the Product Search function to find Pack Inserts and Material Safety Data Sheets.

Roche Diagnostics

Roche Diagnostics GmbH
Roche Applied Science
68298 Mannheim
Germany