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

# cOmplete Lysis-B (2×)

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

**Cat. No. 04 719 930 001**

**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 Protease Inhibitor Cocktail Tablets | • 20 tablets, supplied in *EASYpacks* (foil blisters)<br>• Each tablet is sufficient for a volume of 5 ml solution.<br>• Each tablet contains 3.7 mg EDTA, resulting in a 2 mM EDTA solution in 5 ml. |

### 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×) is intended for the rapid 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 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, cysteine proteases, and metalloproteases.

Lysis-B Reagent purifies soluble proteins and inclusion bodies to nearly homogenous 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 tablets contain EDTA. If the protein of interest is to be purified by IMAC (immobilized metal-chelate affinity chromatography), *e.g.*, Poly-His tagged recombinant proteins, EDTA has to be removed (*e.g.*, by dialysis) prior to the chromatography. Alternatively, the product cOmplete Lysis-B (2×), EDTA-free can be used (see table "Ordering Information").

## 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 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)

❶ 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.

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

❸ 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 (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 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.

### 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 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 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 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.
• 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 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 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 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.
• 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.

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 for every 1 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

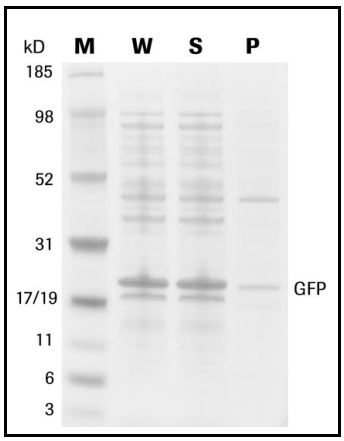


**Fig. 1**: SDS-PAGE analysis and Coomassie blue staining of proteins extracted from *E. coli* BL21 DE3 cells overexpressing GFP.

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 Protease Inhibitor Tablets.

| Protease or protease mixture | Enzyme concentration (µg/ml) | % inhibition after immediate addition to the protease |
|---|---|---|
| Pancreatic extract | 20 | 93% |
| Thermolysin | 0.5 | 97% |
| Trypsin | 0.2 | 87% |
| Papain | 330 | 93% |

One c*O*mplete, Mini 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 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×) 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 (*E. coli* in small volumes). This simple extraction method c*O*mpletely eliminates the need for mechanical disruption (*e.g.*, standard sonification). 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 tablets, provided with this kit, allow the inhibition of a broad spectrum of serine, cysteine and metalloproteases as well as calpains. Due to the optimized composition of the tablets they show excellent inhibitory effects and are therefore very well suited for the protection of proteins isolated from bacteria. c*O*mplete, Mini contains both irreversible and reversible protease inhibitors.

A significant advantage is that the protease inhibitor tablets can be directly dissolved in the protein extraction reagent of the kit. The extracted proteins can be further purified or analyzed in downstream applications. c*O*mplete, Mini 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.

### 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 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 95% 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:<br>Additional information about the current topic or procedure. |
| ⚠ | Important Note:<br>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<br>200 ml | 11 667 157 001<br>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)<br>3 × 20 tablets in a glass vial (for 50 ml each) | 11 697 498 001<br>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<br>Set II: contains 1g Pefabloc SC and 2 × 25 ml PSC protector solution | 11 873 601 001<br>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<br>40 mg | 11 080 733 001<br>11 734 334 001 |
| **Individual Protease Inhibitors** | Aprotinin | 10 mg<br>50 mg<br>100 mg | 10 236 624 001<br>10 981 532 001<br>11 583 794 001 |
| | Bestatin | 10 mg<br>50 mg | 10 874 515 001<br>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<br>10 mg<br>25 mg | 11 585 673 001<br>10 874 523 001<br>11 585 681 001 |
| | Leupeptin | 5 mg<br>25 mg<br>50 mg<br>100 mg | 11 017 101 001<br>11 017 128 001<br>11 034 626 001<br>11 529 048 001 |
| | $\alpha_2$-Macroglobulin | 25 inhibitory units | 10 602 442 001 |
| | Pefabloc SC | 100 mg<br>500 mg<br>1 g | 11 429 868 001<br>11 585 916 001<br>11 429 876 001 |
| | Pepstatin | 2 mg<br>10 mg<br>50 mg | 10 253 286 001<br>11 359 053 001<br>11 524 488 001 |
| | PMSF | 1 g<br>10 g<br>25 g | 10 236 608 001<br>10 837 091 001<br>11 359 061 001 |
| | TLCK – HCl | 100 mg<br>250 mg | 10 874 485 001<br>10 874 493 001 |
| | Trypsin Inhibitor (chicken, egg white) | 1 g | 10 109 878 001 |
| | Trypsin Inhibitor (soybean) | 50 mg<br>500 mg | 10 109 886 001<br>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.

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

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