complete Lysis-B (2x)

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

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

<table>
<thead>
<tr>
<th>Label</th>
<th>Contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysis-B Reagent (2× conc.)</td>
<td>100 ml</td>
</tr>
</tbody>
</table>
| cComplete, Mini Protease Inhibitor Cocktail Tablets | 20 tablets, supplied in EASYpacks (foil blisters)  
• Each tablet is sufficient for a volume of 5 ml solution.  
• 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 cComplete 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
cComplete Lysis-B (2x) 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). cComplete 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 homogeneous levels. The reagent has also been tested for the extraction of proteins from insect cells infected by baculovirus (a sample protocol is provided).

Roche Applied Science

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 cComplete, 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 cComplete to a final concentration of 10 mg/mL. Use a fresh lysozyme solution each time.
• The addition of DNase I to the extraction reagent (fc 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.
• 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 cComplete 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.
2.3 Protocol for Medium-Scale Bacterial Protein Extraction (40 ml bacterial culture, OD\textsubscript{600} nm 1.5 – 3.0)

1. Harvest bacterial cells by centrifugation at approx. 3,000 \times 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\textsubscript{m}plete 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 \times 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 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\textsubscript{m}plete to the suspension and vortex for 1 min.

5. Resuspend the pellet in 20 ml of 1:20 diluted Lysis-B Reagent containing c\textsubscript{m}plete and vortex for 1 min.

6. 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\textsubscript{m}plete to the suspension.
   - Mix by vortexing.

7. Centrifuge inclusion bodies at 27,000 \times g for 15 min.
   - Resuspend the pellet in 100 ml of 1:20 diluted Lysis-B Reagent containing c\textsubscript{m}plete.
   - Vortex briefly.

8. Perform Step 6 two more times.

9. Centrifuge the lysed cells at 27,000 \times g for 15 min. The soluble proteins are separated from the insoluble fraction during centrifugation.

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

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\textsuperscript{*}).
   - Add 0.25 - 0.5 ml of Lysis-B Reagent containing c\textsubscript{m}plete.

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 \times 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 \times 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\textsubscript{m}plete 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 \times 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 cComplete. The extracted proteins were analyzed by SDS-PAGE (5 μg/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.

**cComplete**, 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 cComplete, Mini in Lysis-B Reagent are shown in table 1.

**Table 1**: Inhibition of different proteases by cComplete Protease Inhibitor Tablets.

<table>
<thead>
<tr>
<th>Protease or protease mixture</th>
<th>Enzyme concentration (μg/ml)</th>
<th>% inhibition after immediate addition to the protease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreatic extract</td>
<td>20</td>
<td>93%</td>
</tr>
<tr>
<td>Thermolysin</td>
<td>0.5</td>
<td>97%</td>
</tr>
<tr>
<td>Trypsin</td>
<td>0.2</td>
<td>87%</td>
</tr>
<tr>
<td>Papain</td>
<td>330</td>
<td>93%</td>
</tr>
</tbody>
</table>

One cComplete, 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 cComplete, 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 cComplete 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 cComplete Lysis-B (2×) bacterial protein extraction reagent (Lysis-B Reagent) contains a mild, double-concentrated, non-ionic detergent (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 completely 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 downstream protein purification.

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

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 cComplete, 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. cComplete, 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. cComplete, 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**


**Quality Control**

The inhibitory power of cComplete, 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:

<table>
<thead>
<tr>
<th>Text Convention</th>
<th>Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Numbered Instructions labeled 1, 2, etc.</td>
<td>Steps in a procedure that must be performed in the order listed</td>
</tr>
<tr>
<td>Asterisk *</td>
<td>Denotes a product available from Roche Applied Science</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>🔴</td>
<td>Information Note: Additional information about the current topic or procedure.</td>
</tr>
<tr>
<td>🚩</td>
<td>Important Note: Information critical to the success of the procedure or use of the product.</td>
</tr>
</tbody>
</table>

Abbreviations
In this Instruction Manual the following abbreviations are used:

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Meaning</th>
</tr>
</thead>
<tbody>
<tr>
<td>f.c.</td>
<td>final concentration</td>
</tr>
<tr>
<td>PAGE</td>
<td>polyacrylamide gel electrophoresis</td>
</tr>
<tr>
<td>RT</td>
<td>room temperature</td>
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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 Special Interest Site at:

6.3 Notice to Purchaser

Disclaimer of License
Bacterial protein extraction reagent technology is protected by US Patent 6,174,704.

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