Product Information

# CelLytic™ B Cell Lysis Reagent

For bacterial cell lysis, standard strength

**B7435**

## Product Description

CelLytic™ B is used for the lysis of bacterial cells for the purification of recombinant and wild type proteins. CelLytic™ B contains 40 mM Tris-HCl, pH 8.0, and a proprietary, non-denaturing formulation of zwitterionic detergents. There is no need for special equipment to disrupt cells such as a sonicator or French press. This fast one-step method obtains higher yields than sonication, lysozyme treatment, or other commercially available lysis solutions.

CelLytic™ B can be used to extract soluble proteins, and also to wash away cell debris from inclusion bodies to yield nearly pure protein. CelLytic™ B does not solubilize inclusion bodies.

CelLytic™ B is optimized for the lysis of *E. coli* strain BL21. However, it also works well for DH5α™, JM109, and other similar bacterial cells. Intact fusion proteins have been successfully purified from CelLytic™ B lysates of BL21 *E. coli* cells expressing His-tagged and FLAG®-tagged proteins, using HIS-Select™ and Anti-FLAG® M2 purification resins, respectively. The CelLytic™ B reagent is also compatible with affinity purification of other fusion proteins.

Several theses[1] and dissertations[2-8] cite use of B7435 CelLytic™ B in their research protocols.

## Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

## Reagent

CelLytic™ B is supplied as a ready-to-use solution.

## Reagents and Equipment Required but Not Provided

(Cat. Nos. have been given where appropriate)

- Lysozyme Solution (Cat. No. L3790)
- Benzonase® (Cat. No. E1014)
- Deionized water, or molecular biology grade water (Cat. No. W4502), for dilution of CelLytic™ B
- HIS-Select™ Nickel Affinity Gel (Cat. No. P6611) or other fusion protein purification system
- Protease Inhibitor Cocktails for:
  - Bacterial cells, such as Cat. No. P8465
  - His-tagged proteins, such as Cat. Nos. P8849, S8830, PIC0004
- CelLytic™ IB (Cat. No. C5236)
- Appropriate centrifuge tubes
- Centrifuge

## Storage/Stability

Store the product at room temperature. CelLytic™ B, as supplied, is stable for at least 2 years at room temperature.

## Preparation Instructions

CelLytic™ B is a ready-to-use solution. Additional reagents may be added to suit particular needs. These include salts, protease inhibitors, EDTA (where appropriate; not recommended for purification of His-tagged proteins), or reducing agents such as dithiothreitol or 2-mercaptoethanol.

It is recommended that the entire Technical Bulletin be read prior to use, especially the Reagent Compatibility Chart.

## Procedures

### Trial-Scale Extraction

A small-scale trial extraction should be performed to determine the fraction in which the protein of interest will be found. If this has already been determined, proceed to the Large-Scale Extraction section.

1. A 5 mL culture of the bacterial strain that contains the recombinant protein should be grown under the appropriate conditions for expression.
2. Use 1.5 mL of the bacterial culture with an $OD_{600}$ of 0.5-1.0 and centrifuge the cells at full speed for minutes.
3. Remove the spent medium and resuspend the cell pellet in 0.4 mL of CelLytic™ B.
4. Briefly vortex the solution to resuspend the cell pellet. Mix for 5-10 minutes to ensure full extraction of the soluble proteins.
5. Centrifuge the cell lysate at full speed for 5 minutes to pellet any insoluble material.
6. Carefully remove the soluble protein fraction from the cell debris. Additional extractions may be performed if required. However, this will result in a more dilute soluble protein sample.
7. Analyze the supernatant and the insoluble fraction by SDS-PAGE and/or Western blot to determine which fraction contains the protein of interest. For SDS-PAGE, it is recommended that 5-15 µL of each sample be applied to the gel.

**Note**: If the protein of interest is not found in the soluble portion, it has likely formed inclusion bodies. For the purification/solubilization of inclusion bodies, see the Inclusion Body Purification Procedure, Section C.

### Large-Scale Extraction

This procedure is designed for 1 gram of wet cell paste. This is roughly equivalent to a 250 mL bacterial culture with an $OD_{600}$ of ~2.0. To extract the maximum amount of soluble protein, the CelLytic™ B to cell mass ratio should be 10-20 mL per gram of wet cell paste. Using less CelLytic™ B will give a more concentrated solution, but a smaller amount of total protein will be extracted. Using more CelLytic™ B will not extract any more protein, and instead will only serve to provide a more dilute protein solution.

1. Collect the bacterial cells that express the protein of interest by centrifuging at 5,000 × *g* for 10 minutes.
2. Carefully remove the spent medium from the cell pellet. The cell pellet may be frozen or used fresh. A frozen cell pellet will give a slightly higher yield of protein.
3. Add CelLytic™ B at a ratio of 10-20 mL per gram of cell paste. Mix well to completely resuspend the cells. The following optional reagents may also be added:
   - Lysozyme (final concentration of 0.2 mg/mL) to enhance cell lysis.
   - Benzonase® (final amount of 50 units/mL) to decrease the viscosity of the solution.
   - Protease inhibitors to prevent proteolytic degradation.
4. Incubate the extraction suspension with shaking at room temperature for 10-15 minutes, to extract fully the soluble proteins from the cells.
5. After the cells have been extracted, centrifuge the extract at 16,000 × *g* for 10 minutes to pellet the insoluble material.
6. Carefully remove the supernatant containing the soluble protein fraction. Another round of extraction will yield more soluble protein if required. However, this will result in a more dilute soluble protein sample.
7. Analyze the supernatant and insoluble fraction by SDS-PAGE and/or Western blot to determine which fraction contains the protein of interest. For SDS-PAGE, it is recommended that 5-15 µL of each sample be applied to the gel.

**Note**: CelLytic™ B will **not** solubilize inclusion bodies. For purification of inclusion bodies, see the Inclusion Body Purification Procedure, Section C.

### Inclusion Body Purification

1. Resuspend the cell pellet from the first extraction (Section A, Step 6; or Section B, Step 6) in a volume of CelLytic™ B solution equal to that used for the extraction. Vortex for 1-2 minutes to completely resuspend the cell debris. Add Lysozyme Solution (Cat. No. L3790) to a final concentration of 0.2 mg/mL.
2. Incubate at room temperature for 5-10 minutes to allow the lysozyme to fragment the cell wall.
3. Dilute CelLytic™ B 10-fold by mixing 90 mL of deionized water for every 10 mL of CelLytic™ B.
4. Add 30 mL of the 10-fold diluted CelLytic™ B per gram of paste from the original extraction to the tube. Mix thoroughly.

5. Centrifuge at full speed for 5 minutes to pellet the cell debris again. Save the supernatant for analysis.
6. Resuspend the pellet in a volume of 10-fold diluted CelLytic™ B equal to that was used in Step 4. Vortex to completely resuspend any remaining insoluble material.
7. Centrifuge at full speed for 5 minutes to pellet the inclusion bodies. Save the supernatant for analysis.

**Note**: Steps 5 and 6 may be repeated several times to remove completely any remaining soluble proteins and cell wall material from the inclusion bodies. This wash step should be optimized for the specific protein of interest.

8. Resuspend the washed inclusion bodies (pellet from Step 7) in an equal volume of deionized water or a buffer of choice.
9. Analyze all the saved supernatants and the insoluble fraction by SDS-PAGE and/or Western blot. For SDS-PAGE, it is recommended that 5-15 µL of each sample be applied to the gel.

**Note**: Alternatively, inclusion bodies can be solubilized in CelLytic™ IB (Cat. No. C5236).

## References


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3. Simon-Deckers, Angélique, "Biological effects of manufactured nanoparticles: influence of their characteristics". Institut des Sciences et Industries du Vivant et de l'Environnement, Ph.D. dissertation, p. 230 (2008).
4. Zhou, Chun, "Effect of prostate cancer-related plexin-B1 mutations on cell signalling and function". University College London, Ph.D. dissertation, p. 169 (2009).
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## Troubleshooting Guide

| Problem | Cause | Solution |
|---|---|---|
| Lower than expected protein levels | Cells are not completely lysed. | • Freeze/thaw cells to increase cellular breakage.<br>• Addition of lysozyme (final concentration of 0.2 mg/mL) will aid in protein extraction. |
| | Sample viscosity is too high. | Addition of Benzonase® (final activity of 50 units/mL) will reduce sample viscosity and aid in recovery of soluble extract. |
| | Target protein degraded. | • Addition of protease inhibitors may help reduce target protein degradation.<br>• See Reagents and Equipment Required but Not Provided for recommended protease inhibitor cocktails. |
| | Expression level may be too low. | • Add more inducing agent.<br>• Induce for a longer time period.<br>• Check the construct.<br>• Use another bacterial cell line. |
| | Protein of interest may be insoluble. | Check pellet to ensure protein of interest has not formed inclusion bodies. |

## Reagent Compatibility Chart

| Reagent | Effect | Comments |
|---|---|---|
| Chelating agents (EDTA, EGTA) | Strips metal ions from IMAC resins and chelates essential $Mg^{2+}$ | • EDTA is **not** compatible with the HIS-Select™ line of products. It will chelate metal ions from the affinity gel.<br>• Addition of EDTA to the original cell lysis mixture will chelate metal ions essential for endonuclease activity. This will result in a thick, viscous solution. |
| Protease Inhibitors | Prevent protein degradation | Protease inhibitors may be added to the bacterial cell culture extraction, if desired. |
| 2-mercaptoethanol and dithiothreitol | Reducing Agents | • Can be used at low levels for downstream application to HIS-Select™ products.<br>• Should **not** be used for FLAG® or glutathione resins. |



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