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Product Information

CelLytic™ B Plus Kit

Catalog Numbers **CB0500 and CB0050**
Store CelLytic B Lysis Reagent at Room Temperature
Store remainder of kit at –20 °C

TECHNICAL BULLETIN

Product Description

The CelLytic™ B Plus Kit contains all of the reagents and chemicals necessary to lyse both Gram-negative and difficult to lyse Gram-positive bacteria. The kit also includes protease inhibitors to help prevent the proteolytic breakdown of proteins. The method provided can be used to extract soluble proteins, and can be used to remove cellular debris from inclusion bodies to yield nearly pure protein. Note that for the purification of proteins from inclusion bodies, CelLytic IB (Catalog Number C5236) may also be needed, since CelLytic B does not solubilize inclusion bodies.

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.

Intact fusion proteins have been successfully purified from CelLytic B lysates of BL21 *E. coli* cells expressing histidine-tagged and FLAG fusion proteins using HIS-Select® and ANTI-FLAG® M2 purification resins, respectively. The CelLytic B Plus Kit is also compatible with affinity purification of other fusion proteins.

CB0500 and CB0050 provide reagents sufficient for processing 50 g or 5 g of cell paste, respectively. Fewer grams of cell paste can be processed if proteins are to be extracted from inclusion bodies.

Components for CelLytic B Plus Kit (CB0500)

- CelLytic B, Bacterial Lysis Reagent (Catalog Number B7435) — 500 ml
- Lysozyme Solution (Catalog Number L3790) — 10 × 1.0 ml
- Benzonase® (Catalog Number E1014) — 25,000 units
- Protease Inhibitor Cocktail for Use in Purification of Histidine-tagged Proteins (Catalog Number P8849) — 5 ml

Introductory Size (CB0050)

- CelLytic B, Bacterial Lysis Reagent (Catalog Number B7435) — 50 ml
- Lysozyme Solution (Catalog Number L3790) — 1.0 ml
- Benzonase (Catalog Number E1014) — 5,000 units
- Protease Inhibitor Cocktail for Use in Purification of Histidine-tagged Proteins (Catalog Number P8849) — 1 ml

Reagents and Equipment Required but Not Provided

(Catalog Numbers have been given where appropriate)

- Deionized or molecular biology grade water (Catalog Number W4502), for dilution of the
- CelLytic B Reagent
- HIS-Select Nickel Affinity Gel (Catalog Number P6611) or other fusion protein purification system
- CelLytic IB (Catalog Number C5236)
- Appropriate centrifuge tubes
- Centrifuge

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

It is recommended that the entire technical bulletin be read prior to use, especially the reagent compatibility chart.

Storage/Stability

Remove the CelLytic B Bacterial Lysis Reagent from the kit and store the CelLytic B Bacterial Lysis Reagent at room temperature. Store the rest of the kit at –20 °C. Both the CelLytic B Plus Kit and the CelLytic B Bacterial Lysis Reagent are stable for at least one year if stored properly.

Preparation Instructions
The CelLytic B Plus Working Solution must be prepared immediately before use with the cell paste.

1. Prepare the CelLytic B Plus Working Solution according to the chart below. Adjust the amount of material according to the amount of cell paste to be lysed. The Benzonase has lot specific information on each vial that will determine the volume needed.

Amount of Cell Paste to Lyse (g)	Amount of Each Reagent Needed to make CelLytic B Plus Working Solution			
	CelLytic B Reagent (ml)	Lysozyme (ml)	Protease Inhibitors (ml)	Benzonase (units)
0.5	5	0.1	0.05	250
1	10	0.2	0.1	500
3	30	0.6	0.3	1,500
5	50	1.0	0.5	2,500

Optional: If inclusion bodies need to be purified, in addition to the CelLytic B Plus Working Solution, make a 10-fold dilution of CelLytic B Reagent. Use 90 ml of deionized water for every 10 ml of CelLytic B to yield 100 ml of diluted reagent.

Procedures
A. 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 section on Large Scale Extraction.

1. A five ml culture of the bacterial strain containing 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 2 minutes.

3. Remove the spent medium and resuspend the cell pellet in 0.4 ml of CelLytic B Plus Working Solution.

4. Briefly vortex the solution to resuspend the cell pellet and 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. If desired, the soluble fraction(s) obtained may be applied directly to an affinity resin or other chromatography medium for protein purification.

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.

B. 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. In order to extract the maximum amount of soluble protein, the CelLytic B to cell mass ratio should be 10 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; it 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 Plus Working Solution at a ratio of 10 ml per gram of cell paste. Mix well to completely resuspend the cells.

4. Incubate the extraction suspension with shaking at room temperature for 10–15 minutes to fully extract 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. If desired, the soluble fraction(s) obtained may be applied directly to an affinity resin or other chromatography medium for protein purification.

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.

C. Inclusion Body Purification

1. Resuspend the cell pellet from the first extraction (Section A or B, step 6) in an equal volume of CelLytic B Reagent that was used for the extraction. Vortex for 1–2 minutes to completely resuspend the cell debris. Add the Lysozyme Solution (Catalog Number 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. Prepare a 10-fold diluted CelLytic B solution by mixing 90 ml of deionized water for every 10 ml of CelLytic B Reagent.

4. Add 30 ml of the 10-fold diluted CelLytic B solution per gram of paste from the original extraction to the tube and 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 an equal volume of the 10-fold diluted CelLytic B solution that was used in step 4 and 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. Steps 5 and 6 may be repeated a number of times to completely remove 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 of 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 (Catalog Number C5236).

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Troubleshooting Guide

Problem	Cause	Solution
Lower than expected protein levels	Cells not completely lysed.	Freeze/thaw cells to increase cellular breakage.
		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.
	Expression level may be too low.	• Add more inducing agent. • Induce for a longer time period. • Check the construct. • Use another bacterial cell line.
	Protein of interest may be insoluble.	Check pellet to ensure protein of interest has not formed inclusion bodies.
	Not enough CelLytic B added to cells for full extraction	Use more of the reagent per amount of cells. However, it is not beneficial to add more than 10 ml per gram of wet cell paste