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ProductInformation

CelLytic™ B Plus Kit

Product No. **CB0500** and **CB0050**
Store CelLytic B Lysis Reagent at room temperature
Store remainder of Kit at –20 °C

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 (Product No. C 5236) will also be needed, since CelLytic B does not solubilize inclusion bodies.

The CelLytic B Lysis Reagent is used for the lysis of bacterial cells for the purification of recombinant and wild type proteins. CelLytic B Reagent consists of 20 mM Tris-HCl, pH 7.5, and a proprietary nonionic detergent. There is no need for special equipment such as a sonicator or a French press with this kit.

This kit is compatible with FLAG, histidine and glutathione S-transferase based affinity chromatography protein purification systems. If required for other protein purification systems, the detergent can be removed from the protein by dialysis or ammonium sulfate precipitation. The final purity of the recombinant product is usually higher than that obtained from traditional extraction methods as the proprietary non-ionic detergent eliminates much of the non-specific binding that occurs during chromatography. The mild detergent does not interfere with many enzyme assays or protein assays (See Technical Tips for more information on protein assays).

CB0500 and CB0050 provide reagents sufficient for processing 50 g or 5 g of cell paste, respectively. Fewer grams of cell paste (approx. 14 g in the case of CB0500) can be processed if proteins are to be extracted from inclusion bodies.

Components for CelLytic™ B Plus Kit (CB0500)

- CelLytic B, Bacterial Lysis Reagent, 500 ml, Product No. B 3553
- Lysozyme Solution, 10 x 1.0 ml, Product No. L 3790
- Benzonase, 25,000 units, Product No. E 1014
- Protease Inhibitor Cocktail for (poly)Histidine-tagged proteins, 5 ml, Product No. P 8849

Introductory Size (CB0050)

- CelLytic B, Bacterial Lysis Reagent, 50 ml, Product No. B 3553
- Lysozyme Solution, 1.0 ml, Product No. L 3790
- Benzonase, 5,000 units, Product No. E 1014
- Protease Inhibitor Cocktail for (poly)Histidine-tagged proteins, 1 ml, Product No. P 8849

Materials needed but not provided

- CelLytic IB Reagent for the solubilization of inclusion bodies, Product No. C 5236
- Deionized or molecular biology grade water, e.g. Product No. W 4502, for the dilution of the CelLytic B reagent in the purification of inclusion bodies
- Appropriate centrifuge tubes
- Centrifuge

Precautions and Disclaimer

For laboratory use only. Not for drug, household or other uses.

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. Phase separation and/or precipitation may occur if the CelLytic B Bacterial Lysis Reagent is cold or frozen. The detergent will solubilize once the solution is warmed to room temperature. Both the CelLytic B Plus Kit and the CelLytic B Bacterial Lysis Reagent are stable for at least one year if stored at suggested temperatures.

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.

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 (µl)
0.5	5	0.1	0.05	1
1	10	0.2	0.1	2
3	30	0.6	0.3	6
5	50	1.0	0.5	10

Optional: If inclusion bodies need to be purified, in addition to the CelLytic Working reagent, make a 1:10 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.

Procedure

Purification

This procedure is designed for 1 gram of wet cell paste. This amount of wet cell paste is roughly equivalent to a 250 ml of bacterial culture with an OD_{600} of approximately 2.0.

Note: In order to extract the maximum amount of soluble protein use 10-20 ml of CelLytic B per gram of wet cell paste. Using less CelLytic B will give a more concentrated solution, but a smaller percentage of total protein will be extracted. Using more CelLytic B will not extract more protein; it will only serve to provide a more dilute protein solution.

1. Collect the cells that express the protein of interest by centrifuging at 5,000 x *g* for 10 minutes.

2. Carefully remove the media from the cell pellet. The cell pellet may be frozen or used fresh.

3. Use described volume of CelLytic B Plus Working Solution (See Preparation Instructions above) per gram of cell paste. Mix the sample well to completely resuspend the cells.

4. Incubate the extraction suspension with shaking at room temperature for 10-15 minutes to fully extract the protein from the cells.

5. Centrifuge the extract at 1,900 x *g* for 15 minutes to pellet the insoluble material.

6. Carefully remove the supernatant containing the soluble proteins. Approximately 90 to 95% of the soluble proteins will be found in this fraction. Another round of extraction will yield more soluble protein but it will be more dilute.

7. Optional: If purifying an inclusion body, continue with steps 8-13 of this procedure. If not, continue to the results section.
   Note: CelLytic IB Inclusion Body Solubilization Reagent (Product No. C 5236) is needed to solubilize inclusion bodies, since CelLytic B does not solubilize inclusion bodies.

8. Resuspend the cell debris with 10 ml of CelLytic B Reagent. Mix well to completely resuspend the material. Add 0.2 ml of Lysozyme Stock Solution. Incubate the mixture at room temperature for 5-10 minutes.

9. Add 30 ml of 1:10 diluted (see Preparation Instructions) CelLytic B Reagent to the extract and mix well.

10. Centrifuge the extract at 1,900 x *g* for 15 minutes to collect the inclusion bodies.

11. Carefully remove the supernatant from the inclusion bodies.

12. Resuspend the inclusion bodies in 40 ml of 1:10 diluted CelLytic B.

13. Centrifuge the extract at 1,900 x *g* for 15 minutes to collect the inclusion bodies.

14. Repeat steps 10-12 two more times to further wash the inclusion bodies. Then solubilize the inclusion bodies with CelLytic IB Inclusion Body Solubilization Reagent by following the instructions provided with that product.

Results

The soluble protein fraction is usually clear with a light brown tint. The actual protein concentration will vary according to many different factors such as expression level of the recombinant system, cell type and amount of lysis reagent used.

Technical Tips

1. The CelLytic B Plus Kit is compatible with the FLAG purification system. It has been tested with bacterial cells that express FLAG fusion proteins and is compatible with the ANTI-FLAG M2 agarose affinity gel (Product No. A 2220).

2. The CelLytic B Plus Kit is also compatible with affinity purification using glutathione resin.
3. The CelLytic B Plus Kit is also compatible with purification of histidine containing fusion proteins using the HIS- Select™ Nickel Chelate product line. For example, HIS-Select resin (Product No. P 6611), HIS-Select HF resin (Product No. H 0537) and HIS-Select Nickel Coated HC Plates (Product No. H 1661 and S 5563)

4. CelLytic B Reagent by itself (no lysozyme or benzonase) works well for E. coli strain BL21. It also works with other common E. coli strains such as DH5α and HB 101. The CelLytic B Reagent may be used on other similar bacterial cells. The CelLytic B Plus Kit is compatible with many difficult to lyse Gram positive bacteria such as *Luconostoc, Bacillus, Streptococcus*.

5. It is recommended to use the extracted proteins as soon as possible after extraction. The low ionic strength of the solution and the detergent may lead to protein instability and subsequent protein precipitation of some proteins. The extract may begin to form a haze due to precipitation of various proteins. This haze can be filtered out using a 0.45 or 0.22 µm filter. This precipitation is accelerated when the samples are frozen. Many protein extracts can be stabilized with addition of glycerol (Product No. G 5516) to 40%.

6. The Bradford protein assay (Product No. B 6916) is compatible with CelLytic B. CelLytic B Plus causes minor interference with the bicinchoninic acid (BCA) (Product No. BCA-1), Lowry (Product No. 690-A) and the biuret (Product No. 690-1) kits. However, the use of a suitable blank will give accurate protein values.

References

1. Anai, M. *et al*., J. Biol. Chem. **245**, 767-774 (1970).
2. Suelter, C.H., A Practical Guide to Enzymology. pp. 78-85 (John Wiley & Sons, 1985).

Troubleshooting Guide

Problem	Cause	Solution
Lower than expected protein levels	Cells are not completely broken	Freeze/thaw the cells and/or heat the sample to 37 °C to increase cellular breakage.
	Sample viscosity is too high	Make sure that benzonase is added to the lysis solution.
	Volume of CelLytic B was too small for amount of cell paste used	Use more of the reagent per amount of cells. However, it is not beneficial to add more than 20 ml per gram of wet cell paste.
	Expression level may be too low	Add more inducing agent. Induce for a longer time period. Check the construct. Use another bacterial cell line.
The soluble extract becomes hazy	Not enough CelLytic B was added for the amount of cell paste	Use 10-20 ml of CelLytic B /gram of wet cell paste.
	Sample was frozen for more than a few weeks	Use the extracted protein fraction within 1-2 weeks or remove the detergent through dialysis.
	The samples were not centrifuged at a high enough *g*-force	Centrifuge the extract up to 25,000 x *g* for at least 15 minutes.
	The expressed protein is aggregating	Addition of glycerol to 40-50% may prevent protein precipitation. Alternatively, the proteins can be precipitated from the extract with ammonium sulfate.[2]

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