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ProductInformation

CelLyticä B 10´

Product Code **C8740**
Store at Room Temperature

TECHNICAL BULLETIN

Product Description

CelLytic™ B 10× is a proprietary, non-denaturing formulation of zwitterionic detergents used for the lysis of bacterial cells and extraction of recombinant proteins. CelLytic B 10× contains no buffering components, giving the researcher flexibility to integrate the lysis reagent into their desired system. 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 10× can be used to extract soluble proteins and can also be used to wash away cell debris from inclusion bodies to yield nearly pure protein. CelLytic B 10× does not solubilize inclusion bodies.

CelLytic B 10× 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 10× 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 10× reagent is also compatible with affinity purification of other fusion proteins. CelLytic B 10× may also be used for the lysis of bacterial cells directly in the culture medium for application to affinity purification.

Reagents and Equipment Required but Not Provided

(Product Codes have been given where appropriate)

- Lysozyme Solution (Product Code L3790)
- Benzonase® (Product Code E1014)
- Deionized or molecular biology grade water (Product Code W4502), for the dilution of the CelLytic B 10× reagent
- HIS-Select Nickel Affinity Gel (Product Code P6611) or other fusion protein purification system
- Trizma Pre-set Crystals, pH 8.0 (Product Code T8443)
- Protease Inhibitor Cocktails for:
  Bacterial Cells (Product Code P8465)
  Histidine-tagged Proteins (Product Code P8849)
- CelLytic IB (Product Code C5236)
- Desired buffering components
- 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.

Preparation Instructions

CelLytic B 10× is supplied as a concentrated detergent solution containing no buffering components. This concentrated formulation allows the researcher to use the buffer system that results in maximum solubility and activity of their target protein. Additionally, a buffer can be selected, which will not interfere with the desired downstream applications. CelLytic B 10× should be diluted in an appropriate buffer to the concentration ideal for optimal extraction of the target protein. CelLytic B 10× is compatible with Tris and phosphate buffers. A small aliquot of CelLytic B 10× should be initially diluted into the desired buffer to ensure it does not precipitate the detergents present in CelLytic B 10×.

Storage/Stability

Store the product at room temperature. CelLytic B 10×, as supplied, is stable for at least 2 years at room temperature.

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. The optimal detergent concentration for extraction of the protein of interest can also be determined at this time. It is recommended that 5, 10, and 20-fold dilutions (into the desired buffer) of the CelLytic B 10× concentrate be tested. If the optimal detergent concentration has already been determined, proceed to the Large Scale Extraction Procedure (Section B).

1. Dilute the concentrated CelLytic B 10× (at the various test concentrations) into the desired buffer system.

2. A 10 ml culture of the bacterial strain containing the recombinant protein should be grown under the appropriate conditions for expression.

3. 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. Prepare a cell sample for each concentration of CelLytic B 10× to be tested.

4. Remove the medium and resuspend each cell pellet in 0.4 ml of a different CelLytic B dilution.

5. Briefly vortex the solutions to resuspend the cell pellets and mix for 5-10 minutes to ensure full extraction of the soluble proteins.

6. Centrifuge the cell lysate at full speed for 5 minutes to pellet any insoluble material.

7. 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.

8. 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 maximal amount of the protein of interest, the optimal detergent level should be used. See Section A for determination of optimal detergent concentration.

1. Collect the bacterial cells that express the protein of interest by centrifuging at 5,000 x *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. Dilute CelLytic B 10× with the desired buffer at the optimal detergent concentration.

4. Add the diluted CelLytic B 10× 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:
   a. Lysozyme (final concentration of 0.2 mg/ml) to enhance cell lysis.
   b. Benzonase (final amount of 50 units/ml) to decrease the viscosity of the solution.
   c. Protease inhibitors to prevent proteolytic degradation.

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

6. After the cells have been extracted, centrifuge the extract at 16,000 x *g* for 10 minutes to pellet the insoluble material.

7. 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.

8. 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 10× 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, step 7 or Section B, step 7) in an equal volume of the diluted CelLytic B 10× solution that was used for the extraction. Vortex for 1-2 minutes to completely resuspend the cell debris. Add Lysozyme Solution (Product Code 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. Centrifuge at full speed for 5 minutes to pellet the cell debris again. Save the supernatant for analysis.

4. Resuspend the pellet in an equal volume of CelLytic B 10× diluted 100-fold and vortex to completely resuspend any remaining insoluble material.

5. Centrifuge at full speed for 5 minutes to pellet the cell debris. Save the supernatant for analysis. Steps 4 and 5 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.

6. Resuspend the washed inclusion bodies (pellet from step 5) in an equal volume of deionized water or a buffer of choice.

7. 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 (Product Code C5236).

D. Bacterial Culture Lysis

The following procedure is designed for the lysis and capture of histidine-tagged protein(s) expressed in BL21 *E. coli* cells grown in Terrific Broth medium (Product Code T9179). Factors such as the type of recombinant protein, medium for cell growth, and cell type may affect the purification procedure. These factors should be optimized for each specific affinity system.

1. A culture of the bacterial strain containing the desired recombinant protein should be grown in conditions optimal for protein expression.

2. Dissolve 0.57 grams of Trizma Pre-set Crystals, pH 8.0 (Product Code T8443) into 10 ml of CelLytic B 10× concentrate.

3. Add 1 ml of the Tris-buffered CelLytic B 10× solution per 9 ml of bacterial culture.

4. Addition of lysozyme (at a final concentration of 0.2 mg/ml) can increase the extraction of cellular proteins. Benzonase may be added (at a final concentration of 50 units/ml) to decrease the viscosity of the solution.

6. Incubate at room temperature with gentle mixing for 15 minutes.

7. The cell lysate can now be directly purified with an appropriate affinity gel. This method has been optimized for purification of histidine-tagged proteins with HIS-Select Affinity Gels.

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. Also, addition of EDTA to the original cell lysis mixture will chelate metal ions essential for endonuclease activity, which 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; should not be used for FLAG or glutathione resins.

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. See Reagents and Equipment Required but Not Provided for recommended protease inhibitor cocktails.
	No buffering component added.	CelLytic B 10× is provided as a detergent concentrate, which contains no buffering component. Lysates not containing a buffering component may reduce the amount of soluble cellular and recombinant protein.
	Expression level may be too low.	• Add more inducing agent. • Induce for a longer time period. • Check the construct. • Use another bacterial cell line.
	CelLytic B 10× was diluted to a low final detergent concentration.	CelLytic B 10× should be diluted 10-fold in a desired buffer for typical protein extractions. Some proteins may require a higher detergent concentration for maximum solubility, so in these cases, the CelLytic B 10× solution should only be diluted 5-fold.
	Protein of interest may be insoluble.	Check pellet to ensure protein of interest has not formed inclusion bodies.

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