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Product Information

CelLytic™ Express

Catalog Number **C1990**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

CelLytic™ Express is used to extract proteins from bacterial cells by lysis directly in the culture medium. CelLytic Express consists of a proprietary blend of a detergent and enzymes optimized for in-culture bacterial cell lysis. There is no need for special equipment to disrupt cells such as a sonicator or French press. The one-step extraction method eliminates the need for cell harvest or clarification of lysates prior to purification, allowing for direct affinity adsorption of target proteins to the resin from a total culture extract. In-culture cell lysis routinely results in greater protein yields than traditional extraction methods, and saves time by eliminating cell harvest steps.

Intact fusion proteins have been successfully purified using HIS-Select® and Anti-FLAG® M2 purification resins. When coupled with affinity gels or magnetic beads, the entire culture, extraction, and purification process can be accomplished directly in the culture flask or tube. Fewer sample manipulations and shorter processing time results in a more intact target protein sample, when compared to traditional methods.

CelLytic Express is provided as a ready-to-use, all-in-one formulation, which does not require the addition of separate reagents for protein extraction. It is available in convenient, pre-weighed packages for lysis of 25 mL and 500 mL cultures.

CelLytic Express is optimized for the lysis of *E. coli* strain BL21. However, it works well with other commons strains such as DH5∝ and JM109. CelLytic Express may also be used on other similar bacterial cells. CelLytic Express has been tested with BL21 *E. coli* cells expressing histidine-tagged and FLAG fusion proteins. It should be compatible with affinity purification of other fusion proteins.

Reagents and Equipment Required but Not Provided (Catalog Numbers given where appropriate)

- HIS-Select Cobalt Affinity Gel (Catalog Number H8162)
- HIS-Select Nickel Affinity Gel (Catalog Number P6611)
- Glutathione-Agarose (Catalog Number G4510)
- Protease Inhibitor Cocktails:
  For Bacterial Cells (Catalog Number P8465)
  For Histidine-tagged Proteins (Catalog Number P8849)
  For General Use (Catalog Number P2714)

Precautions and Disclaimer

This product is 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.

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

Preparation Instructions

CelLytic Express is supplied ready-to-use. However, additional reagents may be used depending on the nature of the protein to be isolated. These include salts, protease inhibitors, and reducing agents such as dithiothreitol or 2-mercaptoethanol. The additional reagents should be added into the bacterial culture after dissolving the CelLytic Express, and not by adding them to the dry powder.

Storage/Stability

This product ships on wet ice and storage at –20 °C is recommended. CelLytic Express, as supplied, is stable for at least two years when stored properly.

Procedure

Bacterial Cell Growth

The bacterial strain containing the recombinant protein of interest should be grown in the conditions needed for expression. CelLytic Express is suitable for lysis in a variety of growth media. Although optimized for lysis of cells grown with Terrific Broth (Catalog Number T9179 or T5574), lysis directly in Luria Broth (Catalog Number L3522 or L2542) media is also possible. Terrific Broth and Luria Broth have both been successfully used for in-culture purification of a histidine-tagged protein.

Although intended for use with cell cultures exhibiting an OD_{600} between 0.5 and 6.0, cell cultures with OD_{600} readings as high as 11.0 have been successfully lysed and purified using CelLytic Express. The final lysis solution may appear hazy at very high cell densities, but it can still be directly loaded onto an affinity resin without a clarification step. Additional CelLytic Express can be added to higher density cell cultures to ensure complete lysis and solubilization.

Trial Scale (25 mL Culture) Purification

A small-scale extraction trial should be performed to optimize parameters for downstream purification procedures. These parameters would include the optimal pH for protein binding to the affinity resin.

It is also beneficial to ensure that the protein of interest does not form inclusion bodies. Procedures for the purification of inclusion bodies can be found in the Technical Bulletin for CelLytic B (Catalog Number B7435). CelLytic IB, an inclusion body solubilization solution, is also available (Catalog Number C5236). Because of the high solubilizing power and unique blend of enzymes found in CelLytic Express, purification of inclusion bodies from CelLytic Express lysates may only require a quick wash and pellet recovery step. This procedure should, however, be determined empirically by the researcher for the inclusion body to be purified.

If optimal parameters have already been determined and inclusion bodies are not a concern, one may proceed directly to Large-Scale Purification.

1. Add the entire contents of one bottle (1.26 g) of CelLytic Express lysis powder to the 25 mL culture. For cultures smaller than 25 mL, add 0.05 g of the CelLytic Express lysis powder for each mL of final culture. Mix the culture by inversion to ensure complete suspension of the lysis powder.
2. Incubate the lysis reaction at room temperature for 15–20 minutes, with occasional mixing.
   Note: The cell lysis step may be performed at 37 °C, which typically results in faster lysis.
3. Following incubation, the lysed cell solution should be almost completely transparent.

Although CelLytic Express is intended for lysis followed by immediate purification, cell lysates typically remain fully solubilized for up to 6 hours without any precipitation of proteins or cell debris. Because of the quicker and more efficient processing using CelLytic Express, protease inhibitors are often not needed as supplements. For target proteins exhibiting degradation problems, protease inhibitors can be added to the final lysed cell solution.

Large Scale (500 mL Culture) Purification

The following procedure is designed for the lysis of 500 mL of bacterial culture with an OD_{600} of approximately 2.0. This is roughly equivalent to 2 g of wet cell paste. In order to extract the maximum amount of cell protein, the final OD_{600} reading of the cells to be lysed should fall in the range of 0.5–6.0.

1. Add the contents of one pouch (25.2 g) of CelLytic Express lysis powder to the 500 mL culture. Mix the culture by inversion to ensure complete suspension of the lysis powder.
2. Incubate the lysis reaction at room temperature for 15–20 minutes, with occasional mixing.
   Note: The cell lysis step may be performed at 37 °C, which typically results in faster lysis.
3. Following incubation, the lysed cell solution should be almost completely transparent.

Although CelLytic Express is intended for lysis followed by immediate purification, cell lysates typically remain fully solubilized for up to 6 hours without any precipitation of proteins or cell debris. Due to the quicker and more efficient processing using CelLytic Express, protease inhibitors are often not needed as supplements. For target proteins exhibiting degradation problems, protease inhibitors can be added to the final lysed cell solution.

Downstream Protein Purification

Because of variations in the composition of bacterial media, it may be necessary to adjust the pH of the lysed cell solution, to ensure optimal binding conditions for the target protein and affinity resin. The optimal pH should be determined empirically for each type of affinity purification to be used.

CelLytic Express is optimized for the purification of histidine-tagged proteins using the HIS-Select affinity gels. The optimal pH range is 6.7–7.0 for binding histidine-tagged proteins from CelLytic Express lysates to the HIS-Select affinity gels. Typically, no pH adjustment is required for purifying recombinant proteins on the HIS-Select affinity gels. CelLytic Express has been tested with BL21 *E. coli* cells expressing histidine-tagged and FLAG fusion proteins.

Because of the presence of metal ions in nearly all cell growth media, an observed loss of resin binding capacity may occur when purifying histidine-tagged proteins with the in-culture lysis method. This small loss in binding capacity is due to metal ion exchange between the medium and the resin, and binding capacity can be recovered by regenerating the resin with fresh metal ions. For a procedure describing this method, please refer to the Technical Bulletin for HIS-Select Nickel Affinity Gel (Catalog Number P6611) or HIS-Select Cobalt Affinity Gel (Catalog Number H8162).

CelLytic Express is designed to not require a clarification step before loading the lysed cell solution onto an affinity resin for purification. An optional clarification step can be performed to pellet any insoluble material by centrifugation of the lysed cell solution at 16,000 × *g* for 15 minutes. Prior to centrifugation, a small-scale purification trial should be performed to ensure that the target protein does not form inclusion bodies, which will be found in the insoluble pellet.

The Bradford protein assay (Catalog Number B6916), Bicinchoninic Acid Kit (Catalog Number BCA1), Micro-Lowry (Catalog Number TP0200) and Biuret (Catalog Number B3934) reagents are all compatible with CelLytic Express, but will require the use of a suitable blank to provide accurate protein quantitation.

Reagent Compatibility Chart

Reagent	**Effect**	**Comments**
Chelating agents (EDTA, EGTA)	Strips metal ions from IMAC resins, 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 the 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. Catalog Number P8849 is recommended for histidine-tagged proteins and Catalog Number P8465 for bacterial cells.
Reducing agents (2-mercaptoethanol, dithiothreitol)	Chemical reduction	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
The cell lysate is hazy.	Cell density is too high.	Cell cultures with an OD_{600} of 0.5–6.0 will not form a hazy solution.
		Cell lysate can be clarified by centrifugation at 16,000 × *g* for 15 minutes. Check pellet to ensure target protein has not precipitated.
		Additional CelLytic Express can be added for high density cell cultures to solubilize remaining particulates.
	Incubation time is too short.	Incubate the cell lysate for at least 15 minutes to ensure that all cell components are completely solubilized.
Lower than expected protein levels	Cells are not completely lysed.	Ensure cell extraction is performed for at least 15 minutes to allow for complete lysis of the cells.
	Expression level may be too low.	Add more inducing agent. Induce for a longer time period. Check the construct. Use a different bacterial cell line.
	pH of protein sample may not be optimal for binding.	Due to the composition of cell media, binding to affinity resins is often pH sensitive. CelLytic Express was optimized for lysis in Terrific Broth medium (Catalog Number T9179), and pH adjustments are usually not necessary in this medium. However, when attempting cell lysis and purification in media other than Terrific Broth, a pH adjustment may be necessary. The optimal pH range for HIS-Select purification of CelLytic Express lysates is 6.7–7.0.

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