

## Product Information

### CellLytic™ Express

1 mL Tablets

Catalog Number **C5491**

Storage Temperature  $-20^{\circ}\text{C}$

## TECHNICAL BULLETIN

### Product Description

CellLytic™ Express 1 mL Tablets are used to extract proteins from bacterial cells by lysis directly in the culture medium. CellLytic Express consists of a proprietary detergent and enzyme blend 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 from CellLytic Express lysates using HIS-Select® and Anti-FLAG® M2 purification resins. When coupled with 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.

CellLytic Express 1 mL Tablets are provided as a ready-to-use, all-in-one formulation, which do not require the addition of separate reagents for protein extraction. Each tablet is suitable for the lysis of 1 mL of bacterial culture. CellLytic Express 1 mL Tablets are available in convenient package sizes of 25 and 100 each.

CellLytic Express is optimized for the lysis of *E. coli* strain BL21. However, it works well with other common strains such as DH5α™ and JM109. CellLytic Express may also be used on other similar bacterial cells. CellLytic 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 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 chart.

### Preparation Instructions

CellLytic Express 1 mL Tablets are 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. These additional reagents should be added into the bacterial culture after dissolving the CellLytic Express tablets.

### Storage/Stability

This product ships on wet ice and storage at  $-20^{\circ}\text{C}$  is recommended. CellLytic Express 1 mL Tablets, as supplied, are stable for at least two years when stored at the recommended temperature.

## Procedures

### Bacterial Cell Growth

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

Although intended for use with cell cultures exhibiting an OD<sub>600</sub> between 0.5 and 6.0, cell cultures with OD<sub>600</sub> readings as high as 11.0 have been successfully lysed and purified using CellLytic 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 CellLytic Express can be added to higher density cell cultures to ensure complete lysis and solubilization.

### Trial Scale Purification

A small-scale extraction trial should be performed to optimize parameters for downstream purification procedures. These parameters 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 CellLytic B (Catalog Number B7435). CellLytic 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 CellLytic Express, purification of inclusion bodies from CellLytic Express lysates may only require a quick wash and pellet recovery step. The procedure for inclusion body purification should be determined empirically by the researcher.

1. Add one CellLytic Express 1 mL Tablet for each milliliter of bacterial culture being lysed.
2. Incubate the lysis reaction at room temperature for 15-20 minutes, with occasional mixing/vortexing.  
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 CellLytic 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 CellLytic Express, protease inhibitors are often not needed as supplements. For target proteins susceptible to degradation, 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 the binding conditions are optimal for the target protein and affinity resin. The optimal pH should be determined empirically for each type of affinity purification employed.

CellLytic Express is optimized for the purification of histidine-tagged proteins using the HIS-Select affinity gels or magnetic beads. For binding histidine-tagged proteins from CellLytic Express lysates to the HIS-Select affinity gels, the optimal pH range is 6.7–7.0. Typically, no pH adjustment is required for purifying recombinant proteins on HIS-Select affinity gels. CellLytic Express has been successfully 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. 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).

CellLytic Express is designed so a clarification step is not required before loading the lysed cell solution onto an affinity resin for purification. An optional clarification step can be performed by centrifugation of the lysed cell solution at 16,000 × *g* for 15 minutes to pellet any insoluble material. 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), Lowry (Catalog Number L3540) and Biuret (Catalog Number B3934) reagents are all compatible with CellLytic Express, but require the use of an appropriate blank to provide accurate protein quantification.

#### 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	Reducing agents can be used at low levels for downstream application to HIS-Select products; they 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 $16,000 \times g$ for 15 minutes. Check pellet to ensure target protein has not precipitated. Additional CellLytic 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.
Protein levels are lower than expected.	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. CellLytic Express was optimized for lysis in Terrific Broth medium (Catalog Number T9179), and pH adjustments are usually not necessary in this medium. 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 CellLytic Express lysates is 6.7–7.0.

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