PopLysis™ Bacterial Lysis and Extraction

(Cat. #786-1670, 786-1675)

INTRODUCTION

PopLysis™ Bacterial Lysis and Extraction buffer is part of our PopLysis™ buffer systems. PopLysis™ buffers are based on cocktail of membrane solubilizing detergents optimized to rapidly solubilize and pop open membrane structures allowing cellular proteins to spill out into lysis solution. Bacteria solubilize immediately releasing entire cellular components as soon as PopLysis™ Bacterial Lysis and Extraction buffer come in contact with cells. No mechanical force is required for 100% solubilization and recovery of cellular proteins. PopLysis™ Bacterial Lysis and Extraction buffer is suitable for rapid screening and purification of recombinant proteins.

The cellular proteins are stable in PopLysis™ buffer and suitable for downstream applications such as enzyme assays, chromatography studies, protein folding studies and gel electrophoresis. Furthermore, PopLysis buffers are amine-free formulations making them suitable for applications that require proteins in amine free buffers

Furthermore, depending on the application, additional agents such as reducing agents, chelating agent, and protease inhibitors cocktail may be added into PopLysis™ Bacterial Lysis and Extraction buffer. This reagent has been tested for use with several widely used bacteria including E. coli strains.

PopLysis™ buffers are offered with option to use lysozyme and nuclease are suitable for extracting proteins from approximately 60g wet cell pellet. PopLysis™ Bacterial Lysis and Extraction buffer and kit with lysozyme and nuclease are offered to enhance solubilization and digestion of contaminating nucleic acids respectively.

COMPATIBILITY

PopLysis™ Bacterial Lysis and Extraction buffer is amine free and is compatible with most downstream applications including running various chromatography, gel electrophoresis applications, and protein folding procedures. The buffer absorbs at 280 nm and thus not suitable for measuring protein concentration at 280 nm. Cell lysates prepared with it is compatible with Bicinchoninic Acid (BCA) Protein Assay (Cat. #786-570) and NI™ protein assay (Non-Interfering Protein Assay™, Cat# 786-005).

ITEM(S) SUPPLIED

Description	PopLysis™ Bacterial Lysis and Extraction buffer (Cat. # 786-1670)	PopLysis™ Bacterial Lysis and Extraction Kit (Cat. # 786-1675)
PopLysis™ Bacterial Lysis and Extraction buffer	250 ml	250 ml
LongLife™ Lysozyme [1500U/µl]	-	2 x 1ml

LongLife™ Nuclease [100X]	-	4 x 0.5 ml

STORAGE CONDITION

The kit and buffer are shipped at ambient temperature. Upon arrival store the buffer at 4°C, and LongLife™ Lysozyme and nuclease at -20°C.

ADDITIONAL ITEMS NEEDED

- DTT, or EDTA depending upon application (Optional).
- Protease inhibitors (Optional, ProteaseArrest™, Cat. # 786-330)

IMPORTANT INFORMATION

- Warm the buffer to room temperature before use as at 4°C buffer turns to colloidal suspension.
- PopLysis™ Bacterial Lysis and Extraction buffer is suitable for both freshly pelleted bacterial cells or frozen cells.
- Depending on applications, DTT and EDTA may be added. Add DTT and EDTA to to get final concentrations of 5mM. Avoid EDTA when adding lysozyme to the buffer.
- Use of lysozyme and nuclease is optional to increase the protein extraction efficiency of proteins. Use 10 µl of Longlife™ Lysozyme and 10 µl Nuclease [100X] of per ml of PopLysis™ Bacterial Lysis and Extraction buffer.
- If the inhibition of protease activity is required, add a cocktail of protease inhibitors to prevent protease activities during extraction procedure. We recommend our Bacterial ProteaseArrest™ (Cat. # 786-330).

PROTOCOL

A. Protein extraction from bacterial cells

1. Centrifuge bacterial culture at 5,000 x g for 10 minutes to pellet cells or use the frozen cell pellet.
2. Resuspend the pellet in Poplysis™ buffer (prewarmed at room temperature) with help of pipette or brief vortex to get homogenous suspension. For each 1 gm bacterial pellet use 4 ml of PopLysis™ Bacterial Lysis and Extraction buffer.
3. **Optional:** Add 10 µl LongLife™ Lysozyme and 10 µl LongLife™ Nuclease per 1 ml bacterial suspension.
4. Incubate the cell suspension at room temperature on shaker for 10-15 minutes.
5. Centrifuge the cell lysate at 15,000 x g for 5 minutes to get the soluble proteins in the cell supernatant.

B. Isolation of Inclusion Bodies.

For inclusion bodies isolation, after the lysis step centrifuge the bacterial lysate at 30,000 x g for 30 minutes at 4°C. Collect the inclusion bodies pellet and wash once with

5-fold or PBS. Centrifuge at 30,000 x g for 30 minutes at 4°C. Collect the inclusion bodies for solubilization and re-folding.

TROUBLESHOOTING

Issue	Suggested reason	Possible solution
PopLysis™ Bacterial Lysis and Extraction Buffer has colloidal suspension	Buffer is cold	Warm to room temperature
Protein of interest is not solubilized	Protein of interest is in inclusion bodies	Adjust the expression conditions
		Isolate the inclusion bodies and proceed to inclusion body solubilization
Cell Lysate is viscous	DNase I or nuclease not added	Add LongLife™ Nuclease to the cells in extraction buffer.

RELATED PRODUCTS

Download our Sample Preparation and Protease & Phosphatase Inhibitors, Enzyme & Assays Handbooks.

http://info.gbiosciences.com/complete-protein-sample-preparation-handbook/
http://info.gbiosciences.com/protease-phosphatase-inhibitors-enzymes-assay-handbook

For other related products, visit our website at www.GBiosciences.com or contact us.

www.GBiosciences.com