Protein Extraction and Lysis Buffers (PE LB™)

ABSTRACT

Use of PE LB™ Buffers for Extraction of Carbonic Anhydrase and Alkaline Phosphatase from E. coli, yeast, mouse pancreases and cultured human cells is evaluated.

Lysis and extraction of protein from cellular and tissue samples is the first critical step for biochemical analysis. Selection of lysis and extraction buffers requires considerations such as recovery of the protein of interest as well as stability of biological activity (1,2). G-Biosciences has developed a series of Protein Extraction & Lysis Buffers (PE LB™), which ensure good protein recovery while maintaining biological activity.

The following PE LB™ series offers a wide selection of buffers for lysis and extraction of protein from bacteria, yeast and animal cells and tissues. PE LB™ buffer system is based on a proprietary combination of organic buffering agents, mild non-ionic detergents, and a combination of various salts and agents to enhance extraction of protein and maintain stability of biological activities of the proteins. Depending on application, additional agents such as chelating agents, reducing agents and protease inhibitors may be added in to the PE LB™ buffer system.

The PE LB™ series of buffers is compatible with most downstream applications including running various chromatography, gel electrophoresis applications, and protein folding procedures. It is also compatible for protein estimation with NI™ Protein Assay (A Non-Interfering Protein Assay, Cat. # 786-005).

In this study, PE LB™ buffers are evaluated for the extraction of carbonic anhydrase and alkaline phosphatase from E. coli, yeast, mouse pancreases, and culture human cells. Protocols for the individual PE LB™ buffers were followed.

Bacterial PE LB™

Bacterial-PE LB™ kit (Cat. #786-176) has been specifically developed for the extraction of total soluble proteins and inclusion bodies from bacterial cells. Bacterial-PE LB™ is a proprietary improvement on the lysozyme based lysis method.

Method: Freshly grown E. coli from a 1.5ml culture was used. The bacterial pellet was suspended in PE LB™ and an appropriate amount of *LongLife™* Lysozyme, supplied with the kit, was added to the suspension. Protease cocktail ProteaseArrest™ (Cat. #786-108) was added to prevent proteolysis damage to the proteins. The suspension was incubated in an ice bucket for 30 minutes. After 30 minutes, the suspension was vigorously vortexed and centrifuged to collect a clear lysate for further analysis.

Yeast PE LB™

Yeast-PE LB™ (Cat. #786-178) is a proprietary improvement on the Zymolyase® based spheroplast preparation and extraction of soluble proteins from yeast cells.

Method: Freshly grown yeast from a 1.5ml culture was used. The yeast pellet was suspended in PE LB™ buffer and an appropriate amount of *LongLife™* Zymolyase® (Cat. #786-036), provided with each kit, was added. Protease cocktail ProteaseArrest™ (Cat. #786-108) was added to prevent proteolysis damage to the proteins. The suspension was incubated in an ice bucket for 30 minutes. After 30 minutes, the suspension was vigorously vortexed and centrifuged to collect a clear lysate for further analysis

Mammalian Cell-PE LB™

Mammalian Cell-PE LB™ (Cat. #786-180) is useful for extraction of total soluble proteins from mammalian cultured cells.

Method: Freshly grown 5 million Jurkat cells were used in this study. The cell pellet was suspended in 0.5ml PE LB™. Protease cocktail, ProteaseArrest™ (Cat. #786-108) was added into PE LB™ to prevent proteolysis damage to the proteins. The cell suspension was vortexed and pulled up and down the pipetor a few times. The suspension was centrifuged and a clear lysate was collected for analysis.

Tissue PE LB™

Tissue-PE LB™ (Cat. #786-181) has been specifically developed for the extraction of total soluble protein from animal tissues.

Method: 50mg mouse pancreas tissue was used in this study. The tissue sample was homogenized in 0.5ml PE LB™. Protease cocktail, ProteaseArrest™ (Cat. #786-108) was added into PE LB™ to prevent proteolysis damage to the proteins. The homogenate was centrifuged and a clear lysate was collected for analysis.

RESULTS AND DISCUSSION

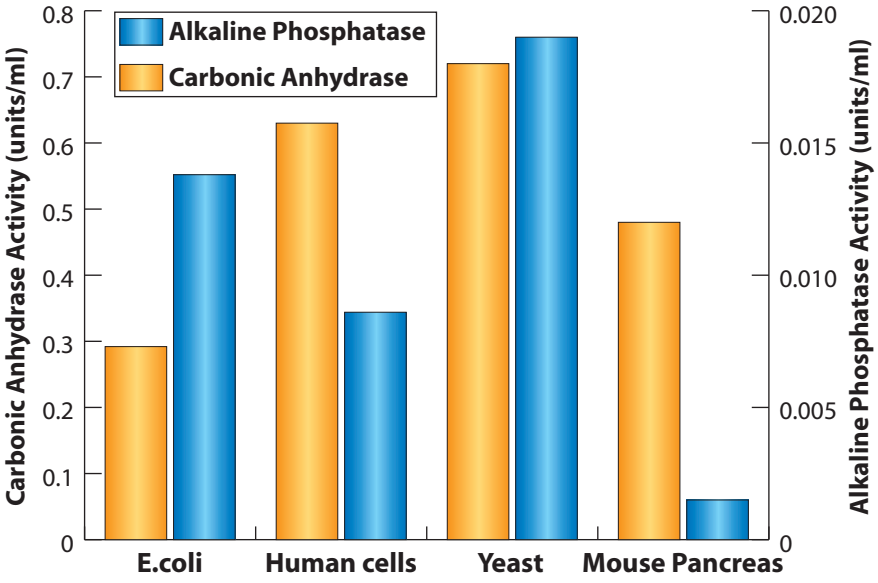

Figure 1 Figure 1: PE LB™ System maintains the biological activity of proteins. Extraction of carbonic anhydrase or alkaline phosphatase from E.coli, human cells, yeast and mouse pancreas with Bacterial, Mammalian Cell, Yeast and Tissue PE LB™ respectively. The resulting lysates were submitted to enzyme assays and both enzymes retain their biological activity.

The results of carbonic anhydrase and alkaline phosphatase activities are shown in Figure 1. Bacterial-PE LB™ eliminates the need for laborious mechanical lysis of bacterial cells and removal of nucleic acids. Bacterial-PE LB™ allows extraction of soluble proteins and concurrent removal of nucleic acids released during cell lysis. The lysis by Bacterial-PE LB™ eliminates viscosity build up.

Yeast-PE LB™ buffer kit provides a simple and versatile method of yeast protein extraction and eliminates the need of glass beads for lysis of yeast cells. Yeast-PE LB™ buffer eliminates viscosity build up due to the release of genomic nucleic acids, allowing effective clarification of the lysate.

Mammalian Cell-PE LB™ and Tissue-PE LB™ are simple to use, quickly extract proteins, and release carbonic anhydrase and alkaline phosphatase activities.

The protein extract prepared by using PE LB™ buffers are suitable for enzyme studies and other downstream applications such as protein purification, electrophoresis, immuno-assays, and so forth.

CONCLUSION

Extraction & lysis buffers (PE LB™) ensure good recovery of carbonic anhydrase and alkaline phosphatase activities while maintaining biological activity.

ORDERING INFORMATION

Cat. #	Description/ Size
786-176	Bacterial-PE LB™ Kit with Lysozyme/ 100 x 50µl cell pellets
786-177	Bacterial-PE LB™ Buffer/ 500ml
786-178	Yeast-PE LB™ Kit with Zymolyase®/ 100 x 50µl cell pellets
786-179	Yeast-PE LB™ Buffer/ 500ml
786-180	Mammalian-PE LB™ Buffer/ 500ml
786-181	Tissue-PE LB™ Buffer/ 500ml

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