cOmplete Lysis Kits: Rapid Protein Extraction and Simultaneous Protection Against Proteases

Cordula Nemetz, Claudia Vorwerk, and Claudia Kirr*
Roche Applied Science, Penzberg, Germany
**Corresponding author: cordula.nemetz@roche.com*

Introduction

Sample preparation is an essential step for all three main areas of protein analysis, namely expression proteomics, structural proteomics, and functional proteomics. As mechanical, chemical, or enzymatic methods can directly interfere with the protein's activity and integrity, a gentle way of efficiently extracting proteins from cells is essential. The use of mild detergents to disrupt cells displays a very simple, effective, and convenient method compared with commonly used harsh treatments. The ready-to-use cOmplete Lysis Reagents enables the time efficient cell lysis of a high number of reactions in parallel (*e.g.*, in a microtiter plate) unlike traditional methods like sonification. By combining lysis reagents with cOmplete Protease Inhibitor Cocktail Tablets, a multitude of proteases can be inhibited at the time of lysis, avoiding degradative conditions. This allows a high level of standardization and reliability, which is necessary for effective and successful proteome analysis. The cOmplete Lysis-B (2x), -M, and -Y Kits contain an efficient and gentle Lysis Reagent and the cOmplete Protease Inhibitor Cocktail Tablets. The kits are also available as EDTA-free version.

Rapid and Effective Lysis of Bacterial Cells and Concurrent Protease Inhibition

The Lysis-B Reagent in the cOmplete Lysis-B Kit is a two-fold concentrated protein extraction reagent, which allows cell lysis in a smaller volume. It is a ready-to-use solution, a further dilution is not necessary. It consists of non-ionic detergents that enable efficient and gentle lysis of bacterial cells and insect cells.

The Lysis-B Reagent solution was used together with the cOmplete Protease Inhibitor Cocktail Tablets of the kit for the extraction of green fluorescent protein (GFP) overexpressed in *Escherichia coli* BL21 DE3 pLysS bacterial cells. The cells were harvested at an OD_{600} of 1.5–2.0 by centrifugation and resuspended in Lysis-B Reagent containing cOmplete Protease Inhibitor Tablets (1 tablet/5 ml lysis reagent). Figure 1a shows the SDS-gel analysis of the whole protein fraction, the supernatant fraction, and the pellet fraction stained with Coomassie Blue. Typically, 3 mg/ml–4 mg/ml of protein are obtained from *E. coli* BL21 cells as determined via Bradford protein assays (Figure 1b). For the standard curve of the Bradford protein assay, bovine serum albumin was

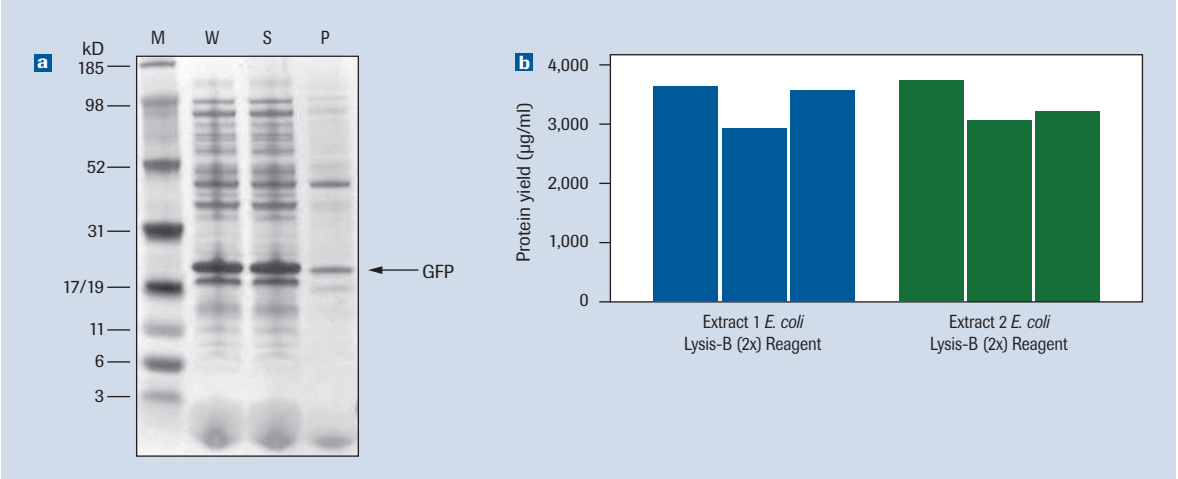

Figure 1: Lysis of prokaryotic cells. (a) SDS-PAGE analysis of proteins extracted from *Escherichia coli* cells. BL21 DE3 pLysS cells were harvested and resuspended in the presence of cOmplete Protease Inhibitor Cocktail Tablets. The extracted proteins were analyzed by SDS-PAGE and Coomassie Blue staining (5 µl/lane). M=marker; W=whole fraction; S=supernatant fraction; P=pellet fraction. **(b)** Lysis efficiency of Lysis-B Reagent. BL21 cells were harvested in Lysis-B Reagent following the instructions of the user manual. The yields of the whole protein fractions from two different lysis reactions were determined in triplicate by a Bradford protein assay (50 µl protein extract + 2.5 ml Bradford reagent).

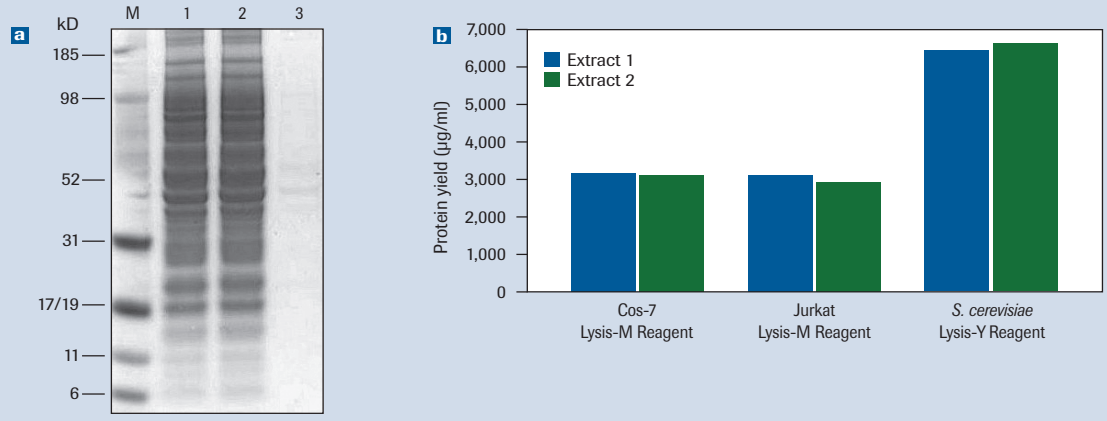

Figure 2: Lysis of eukaryotic cells. (a) SDS-PAGE analysis of proteins extraced from eukaryotic cells. Cos-7 cells at confluency were harvested in Lysis-M reagent. The extracted proteins were analyzed by SDS-PAGE (10 µl probe/lane). M: Marker; Lane 1: whole protein fraction; lane 2: supernatant fraction; lane 3: pellet fraction. **(b)** Lysis efficiency of c*O*mplete Lysis-M Reagent and c*O*mplete Lysis-Y Reagent. Cos-7 cells and Jurkat cells at confluency were harvested in Lysis-M Reagent exactly following the instructions of the user manual. 130 mg of *Saccharomyces cerevisiae* cell pellet were resuspended in 500 µl of Lysis-Y reagent following the instructions of the user manual. All yields of the whole protein fractions from two different lysis reactions were determined after 1:5 dilution in water in duplicate by a Bradford assay (50 µl protein extract + 2.5 ml Bradford reagent).

dissolved in Lysis-B Reagent diluted 1:5 in water. Likewise, the whole bacterial protein extracts were diluted 1:5 in water prior to the determination of the protein yield.

Efficient Lysis of Eukaryotic Cells and Simultaneous Inhibition of Proteases

The combination of detergents of Lysis-M and –Y Reagents allows highly efficient extraction of proteins from cultured adherent mammalian cells, from mammalian cells in suspension (Lysis-M Reagent), and from cells of various yeast strains, as well as from different bacterial cells [*e.g., Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Bacillus subtilis*, and *E. coli* and a variety of gram-positive bacteria (Lysis-Y Reagent)].

The Lysis-M ready-to-use solution was used together with the c*O*mplete Protease Inhibitor Cocktail Tablets of the kit for the lysis of Cos-7 monkey kidney cells. The cells were harvested at confluency and resuspended in Lysis-M reagent containing c*O*mplete Protease Inhibitor Tablets (1 tablet/10 ml lysis reagent). In Figure 2a, a typical SDS-PAGE analysis of the whole protein, the supernatant, and the pellet fraction is shown after Coomassie Blue staining.

Whole protein yields obtained from eukaryotic cells were determined via Bradford protein assay. All cell extracts were diluted 5x in water previous to the Bradford assay. The protein yields obtained from confluent Cos-7 cells, Jurkat cells, and yeast *S. cerevisiae* cells are shown in Figure 2b.

Benefits:

- Save time with ready-to-use reagents for easy, fast, and gentle lysis of bacterial, insect, mammalian, and yeast cells.
- Obtain high yields of extracted proteins.
- Ensure protein integrity by using reagents that do not denature or interact with proteins.
- Achieve non-toxic, effective protection of proteins against a multitude of proteases (serine proteases, cysteine proteases, and metalloproteases).
- Employ reagents that are compatible with downstream assays.

For more information on c*O*mplete Lysis Kits and our other products for protease inhibition, visit: www.roche-applied-science.com/proteaseinhibitor. ■

Order INFO

Product	Pack Size	Cat. No.
c*O*mplete Lysis-B (2x)	100 ml lysis reagent and 20 tablets*	04 719 930 001
c*O*mplete Lysis-B (2x), EDTA free	100 ml lysis reagent and 20 tablets**	04 719 948 001
c*O*mplete Lysis-M	200 ml lysis reagent and 20 tablets*	04 719 956 001
c*O*mplete Lysis-M, EDTA-free	200 ml lysis reagent and 20 tablets**	04 719 964 001
c*O*mplete Lysis-Y	200 ml lysis reagent and 20 tablets*	04 719 972 001
c*O*mplete Lysis-Y, EDTA-free	200 ml lysis reagent and 20 tablets**	04 719 999 001

* c*O*mplete, Mini, Protease Inhibitor Cocktail Tablets, ** c*O*mplete, Mini, EDTA-free, Protease Inhibitor Cocktail Tablets.