



For life science research only. Not for use in diagnostic procedures. FOR *IN VITRO* USE ONLY.

# cOmplete Lysis-Y

Reagent set for highly efficient protein extraction from yeast cells by rapid lysis and concurrent protection of extracted proteins against a multitude of proteases

Cat. No. 04 719 972 001

Version January 2006

Store at +15 to +25°C

## 1. What this Product Does

### Number of Reactions

The set is designed for the lysis of 40 – 80 g of wet yeast cell pellet.

### Kit Contents

Label	Contents
Lysis-Y Reagent	200 ml
cOmplete, Mini Protease Inhibitor Cocktail Tablets	<ul style="list-style-type: none"><li>• 20 tablets, supplied in EASYpacks (foil blisters).</li><li>• Each tablet is sufficient for a volume of 10 ml solution.</li><li>• Each tablet contains 3.7 mg EDTA, resulting in a 1 mM EDTA solution in 10 ml.</li></ul>

### Storage and Stability

If stored at room temperature the kit is stable through the expiration date printed on the label.

### Application

Due to the complex and stable cell wall, yeast protein extraction is difficult and time-consuming. The cOmplete Lysis-Y yeast cell protein extraction reagent allows effective and rapid cell lysis of yeast cells (*e.g.*, *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Pichia pastoris*) in only 20 minutes, combined with simultaneous inhibition of protease activity in the cell lysate. It can also be used for lysis of *E. coli* and *Bacillus subtilis* cells. Proteases are released during the extraction of proteins from yeast or bacteria cells, resulting in rapid degradation of proteins (1). cOmplete Lysis-Y enables highly efficient protein extraction from yeast cells and the simultaneous inhibition of a multitude of proteases, including serine proteases, cysteine proteases, and metalloproteases.

Lysis-Y Reagent effectively lyses freshly harvested and frozen yeast cells. The growth temperature, growth rate and the culture medium can have a significant effect on the number of harvested cells. Therefore, the application of different volumes of Lysis-Y Reagent for various cell pellet (wet cell paste) weights is described in the protocol.

Ⓞ cOmplete, Mini tablets contain EDTA. If the protein of interest is to be purified by IMAC (immobilized metal-chelate affinity chromatography), *e.g.*, Poly-His tagged recombinant proteins, EDTA has to be eliminated (*e.g.*, by dialysis) prior to the chromatography. Alternatively, the product cOmplete Lysis-Y, EDTA-free can be used (see table "Ordering Information").

## 2. How To Use this Product

### 2.1 Before You Begin

#### General Remarks

Lysis-Y Reagent is suited for the extraction of proteins from *Saccharomyces cerevisiae* cells harvested in log-phase growth, as well as from plateau-phase growth, in rich or synthetic defined media. To effectively lyse *Schizosaccharomyces pombe* cells, a culture grown in synthetic defined media such as Edinburgh Minimal Media (EMM). Harvest the cells during log-phase growth to obtain optimal lysis of cells grown in rich media such as YES. Increase temperature to 45°C during lysis to increase protein yield from *S. pombe* cultures grown past log-phase.

*Bacillus subtilis* strains that are unable to sporulate may be grown to saturation before cells being harvested. If, by contrast, strains are used that can produce spores, harvest the bacteria during log-phase growth because Lysis-Y Reagent will not lyse *B. subtilis* spores.

#### Safety precautions

Observe the usual precautions to be taken when handling chemicals.

⚠ Consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

⚠ **Do not eat the tablets.**

#### Preparation of Working Solutions

The tablets can be added directly to the provided Lysis-Y Reagent. One cOmplete, Mini tablet is sufficient for the inhibition of the proteolytic activity in 10 ml Lysis-Y Reagent. Dissolve the tablet in 10 ml of the provided Lysis-Y Reagent by incubating for 2 min at RT, afterwards vortex shortly.

### 2.2 Protocol

- 1 Harvest yeast cells by centrifugation at approx.  $3,000 \times g$  (*e.g.*, 5,000 rpm for Beckman JA-20 rotor) for 5 min at 4°C.
  - ⚠ Cell pellets can be frozen at –15 to –25°C or –80°C before lysis or immediately lysed.
- 2
  - Remove the supernatant and resuspend the pellet in an appropriate amount of Lysis-Y Reagent containing cOmplete as indicated in table 1.
  - Pipet up and down or vortex gently until the mixture is homogeneous.
- 3 Shake the mixture at RT for 20 min.
- 4 Centrifuge at 14,000 rpm for 10 min to pellet any insoluble proteins and cell debris.
  - Ⓞ Expect to recover more than 90% of the soluble proteins from the first extraction. An additional extraction is not usually required, but it might help increase the yield of soluble proteins.

- 5 • Further analyze the protein-containing supernatant (*i.e.*, lysate) in downstream applications, *e.g.*, purification and/or protein concentration determination.
- Store the protein solution short-term at -15 to -25°C (stability is prolonged by storage at -70°C).
- ⚠ Since lysis-Y Reagent contains detergent, it is not compatible with protein assays that are incompatible with detergents. Also, there is no guarantee that a particular protein will retain optimal activity in the presence of Lysis Reagent-Y.

**Table 1:** Volume of Lysis-Y Reagent containing cOplete to add per milligram of yeast cell pellet.

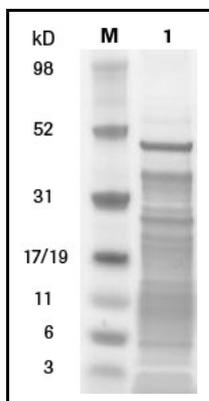
Wet Yeast Cell Pellet Weight (mg)	Lysis-Y Reagent containing cOplete Volume (μl)
50	125 - 250
250	625 - 1,250
500	1,250 - 2,500

### 3. Typical Result

*Saccharomyces cerevisiae* cells were resuspended in Lysis-Y Reagent. The extracted proteins were analyzed by SDS-PAGE (10 μl/lane).

M: marker

lane 1: lysis in the presence of cOplete



**Fig. 1:** SDS-PAGE analysis and Coomassie blue staining of proteins extracted from *Saccharomyces cerevisiae* cells.

cOplete, Mini Protease Inhibitor Cocktail Tablets were dissolved in Lysis-Y Reagent and maintained full functionality for inhibition of a multitude of proteases.

Typical values for the inhibition of different proteases and protease mixtures by cOplete, Mini in Lysis-Y Reagent are shown in table 2.

**Table 2:** Inhibition of different proteases by cOplete Protease Inhibitor Tablets.

Protease or protease mixture	Enzyme concentration (μg/ml)	% inhibition after immediate addition to the protease
Pancreatic extract	20	83%
Thermolysin	0.5	84%
Trypsin	0.2	93%
Papain	330	92%

One cOplete, Mini tablet was added per 10 ml Lysis-Y Reagent. Proteolytic activity was determined with the Roche Applied Science Universal Protease Substrate\* (casein, resorufin-labeled). When extractions or single-step isolations are necessary in the acid pH range, simply include pepstatin\* along with cOplete, Mini tablets to ensure aspartic (acid) protease inhibition. All experiments were performed at room temperature.

\* available from Roche Applied Science

### 4. Troubleshooting

Observation	Possible Cause	Recommendation
Low protein yield	Suboptimal growth conditions	Start from a fresh culture. Harvest cells in log phase
Low protein yields from <i>Schizosaccharomyces pombe</i>	Suboptimal growth conditions	<i>Schizosaccharomyces pombe</i> cells should be grown from cells grown in synthetic defined media ( <i>e.g.</i> , EMM). If cells are grown in rich media ( <i>e.g.</i> , YES), harvest cells during log-phase.

### 5. Additional Information on this Product

#### Product Description

cOplete Lysis-Y protein extraction reagent for yeast cells contains a mild detergent formulation in buffer solution (pH 7.4). This simple extraction method allows very efficient and gentle extraction of proteins from yeast cells and eliminates the need for mechanical disruption (*e.g.*, using glass beads). Efficient lysis occurs in only 20 minutes at room temperature. The protein yields obtained with this kit are two times higher compared to those obtained by using glass beads.

Lysis-Y Reagent has been successfully used to extract soluble proteins from *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Bacillus subtilis* and *Escherichia coli* as well as a variety of gram positive bacteria.

Proteases are ubiquitous in all living cells. As soon as cells are disrupted, proteases are released and can quickly degrade any protein. This can drastically reduce the yield of protein during isolation and purification. The cOplete, Mini tablets provided with this kit allow the inhibition of a broad spectrum of serine, cysteine and metalloproteases as well as calpains. Due to the optimized composition of the tablets they show excellent protease inhibition effects and are therefore very well suited for the protection of proteins isolated from yeast cells. cOplete, Mini contains both irreversible and reversible protease inhibitors. As a main advantage, the protease inhibitor tablets can be directly dissolved in the Lysis-Y protein extraction reagent. The extracted proteins can be further purified or analyzed in downstream applications. cOplete, Mini tablets eliminate the time-consuming search for the right protease inhibitor. The ready-to-use water-soluble, non-toxic tablets work optimally in combination with the Lysis-Y Reagent.

#### References

- 1 North, MJ. (1969) in: Proteolytic Enzymes - A Practical Approach (Beynon, PJ. & Bond, JS. eds.), IRL press Oxford, pp. 117-119.
- 2 Beynon RJ, Bond JS. (1986) Catabolism of intracellular protein: molecular aspects. *Am J Physiol.*, **251** (2 Pt 1), 141-52.

#### Quality Control

The inhibitory power of cOplete, Mini has been demonstrated with many proteases and protease mixtures. In these experiments substantially higher concentrations of proteases were used compared to the concentration usually present in extracts. The inhibitory activity of each lot is tested with a concentrated pancreas extract and a concentrated pronase solution. The proteolytic activities are thereby typically inhibited by 95% after one hour (detection with Universal Protease Substrate, casein, resorufin-labeled\*).

The efficiency of cell lysis using Lysis-Y Reagent is determined for each lot by functional testing.

## 6. Supplementary Information

### 6.1 Text Conventions

To make information consistent and memorable, the following text conventions are used in this package insert:

Text Convention	Use
Numbered Instructions labeled ❶, ❷, etc.	Steps in a procedure that must be performed in the order listed
Asterisk *	Denotes a product available from Roche Applied Science

### Symbols

In this package insert the following symbols are used to highlight important information:

Symbol	Description
❶	Information Note: Additional information about the current topic or procedure.
⚠	Important Note: Information critical to the success of the procedure or use of the product.

### Abbreviations

In this Instruction Manual the following abbreviations are used:

Abbreviation	Meaning
f.c.	final concentration
min	minute(s)
PAGE	polyacrylamide gel electrophoresis
RT	room temperature

### 6.2 Changes to Previous Version

Store kit at room temperature.

### 6.3 Ordering Information

Roche Applied Science offers a large selection of reagents and systems for life science research. For a complete overview of related products and manuals, please visit and bookmark our home page [www.roche-applied-science.com](http://www.roche-applied-science.com).

For additional information on protease inhibition, please visit our Special Interest Site at: [www.roche-applied-science.com/proteaseinhibitor](http://www.roche-applied-science.com/proteaseinhibitor)

	Product	Pack Size	Cat. No.	
<b>Complete Lysis</b>	Lysozyme	10 g	10 837 059 001	
	TriPure Isolation Reagent	50 ml 200 ml	11 667 157 001 11 667 165 001	
	DNase I from bovine pancreas	100 ml sterile	11 284 908 001	
	DNase I recombinant	2 × 10,000 U	04 536 282 001	
	<b>cO/mplete Protease Inhibitor Cocktail Tablets in EASYpacks</b>	cO/mplete	20 tablets in foil blisters (for 50 ml each)	04 693 116 001
cO/mplete, Mini		30 tablets in foil blisters (for 10 ml each)	04 693 124 001	
cO/mplete, EDTA-free		20 tablets in foil blisters (for 50 ml each)	04 693 132 001	
cO/mplete, Mini, EDTA-free		30 tablets in foil blisters (for 10 ml each)	04 693 159 001	
<b>cO/mplete Protease Inhibitor Cocktail Tablets in glass vials</b>		cO/mplete	20 tablets in a glass vial (for 50 ml each) 3 × 20 tablets in a glass vial (for 50 ml each)	11 697 498 001 11 836 145 001
		cO/mplete, Mini	25 tablets in a glass vial (for 10 ml each)	11 836 153 001
		cO/mplete, EDTA-free	20 tablets in a glass vial (for 50 ml each)	11 873 580 001
<b>Kits and Sets</b>		cO/mplete, Mini, EDTA-free	25 tablets in a glass vial (for 10 ml each)	11 836 170 001
		Pefabloc SC PLUS	Set I: contains 100 mg Pefabloc SC and 5 ml PSC protector solution Set II: contains 1g Pefabloc SC and 2 × 25 ml PSC protector solution	11 873 601 001 11 873 628 001
<b>Individual Protease Inhibitors</b>		Protease Inhibitor Set	Small quantities of 10 most commonly used protease inhibitors	11 206 893 001
	Universal Protease Substrate (Casein, resorufin-labeled)	15 mg 40 mg	11 080 733 001 11 734 334 001	
	Aprotinin	10 mg 50 mg 100 mg	10 236 624 001 10 981 532 001 11 583 794 001	
	Bestatin	10 mg 50 mg	10 874 515 001 11 359 070 001	
	Calpain Inhibitor I	25 mg	11 086 090 001	
	Calpain Inhibitor II	25 mg	11 086 103 001	
	Chymostatin	10 mg	11 004 638 001	

Product	Pack Size	Cat. No.
E-64	5 mg	11 585 673 001
	10 mg	10 874 523 001
	25 mg	11 585 681 001
Leupeptin	5 mg	11 017 101 001
	25 mg	11 017 128 001
	50 mg	11 034 626 001
	100 mg	11 529 048 001
α <sub>2</sub> -Macroglobulin	25 inhibitory units	10 602 442 001
Pefabloc SC	100 mg	11 429 868 001
	500 mg	11 585 916 001
	1 g	11 429 876 001
Pepstatin	2 mg	10 253 286 001
	10 mg	11 359 053 001
	50 mg	11 524 488 001
PMSF	1 g	10 236 608 001
	10 g	10 837 091 001
	25 g	11 359 061 001
TLCK - HCl	100 mg	10 874 485 001
	250 mg	10 874 493 001
Trypsin Inhibitor (chicken, egg white)	1 g	10 109 878 001
Trypsin Inhibitor (soybean)	50 mg	10 109 886 001
	500 mg	10 109 894 001
Buffers in a Box, Pre-mixed PBS Buffer, 10×	4 l	11 666 789 001

### Buffers

### 6.4 Notice to Purchaser

#### Trademarks

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