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For life science research only.
Not for use in diagnostic procedures.

cOmplete Lysis-M, EDTA-free

Reagent set for highly efficient protein extraction from mammalian cells by rapid lysis and simultaneous protection of extracted proteins against a multitude of proteases. Suitable for downstream purification using IMAC.

Cat. No. 04 719 964 001

Version 04

Content version: August 2018

Store at +15 to +25°C

1. What this Product Does

Number of Reactions

The set is designed for the lysis of approximately 100 g of mammalian cells.

Kit Contents

Label	Contents
Lysis-M Reagent	200 ml
cOmplete, Mini, EDTA-free Protease Inhibitor Cocktail Tablets	• 20 tablets, supplied in *EASYpacks* (foil blisters) • Each tablet is sufficient for a volume of 10 ml solution.

Storage and Stability

If stored at +15 to +25°C the kit is stable until the expiration date printed on the label.

Application

cOmplete Lysis-M EDTA-free is intended for the efficient and gentle extraction of proteins from both the cytoplasm and the nucleus of cultured mammalian cells. Efficient lysis of mammalian cells occurs in only 5 min at +15 to +25°C, eliminating the need for scraping, sonication or freeze-thaw cycles. cOmplete Lysis-M EDTA-free extraction reagent for mammalian cells contains a mild detergent in 25 mM bicine buffer (pH 7.6). The protein yields obtained with this kit are significantly higher compared to those obtained using sonification.

Lysis-M Reagent is compatible with many different applications, including reporter assays (*e.g.*, β-galactosidase, luciferase, chloramphenicol acetyltransferase), immunoassays (*e.g.*, Western blots, ELISAs, RIAs), protein assays (*e.g.*, protein kinase A, protein kinase C, and tyrosine kinase), and protein purification. Furthermore, the cell lysate is compatible with protein assays such as Coomassie staining and BCA (2'-Benzoyloxycinnamaldehyde) protein assays and the reagent can be removed by dialysis.

cOmplete, Mini, EDTA-free tablets are employed to stabilize those extracts where the stability or activity of metal-containing proteins must not be affected. Since EDTA interferes with IMAC (immobilized metal affinity chromatography), cOmplete, Mini, EDTA-free tablets are preferentially used in the isolation process of Poly-His tagged fusion proteins or subsequent assays. cOmplete, Mini, EDTA-free tablets efficiently inhibit a wide range of serine and cysteine proteases, but not metalloproteases.

2. How To Use this Product

2.1 Before You Begin

General Remarks

For adherent mammalian cells the maximum cell lysis without cell scraping can be obtained by using the volumes of Lysis-M Reagent specified in Table 1. Estimate the volume of cells in case where it is unknown to calculate the required volume of Lysis-M. For example, 2×10^6 of HeLa cells is equivalent to 20 mg of cells (~10 µl of a packed cell volume) and requires 200 µl of Lysis-M Reagent. A smaller volume of Lysis-M Reagent may be used if more concentrated cell extracts are preferred. In this case, however, the cells must be scraped for maximum recovery.

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 cOmplete, Mini, EDTA-free tablets can be added directly to the provided Lysis-M Reagent.
- One cOmplete, Mini, EDTA-free tablet is sufficient for the inhibition of the proteolytic activity in 10 ml Lysis-M Reagent. Dissolve the tablet in 10 ml of the provided Lysis-M Reagent by incubating for 2 min at +15 to +25°C, afterwards vortex shortly.

2.2 Protocol for Lysis of Adherent Mammalian Cells

1. • Remove (decant) culture media from the adherent cells grown in monolayer culture.
   • Optional: Wash cells once in washing buffer (*e.g.* PBS*) if the culture medium contains reagents that could interfere with subsequent protein analysis.
2. • Add the appropriate amount of Lysis-M Reagent containing cOmplete, EDTA-free tablet to each plate or well (see table 1 below).
   • Incubate for 5 min at RT with gentle shaking.
3. • Collect the cell lysate.
   The lysate can be used directly for analysis in the presence of the cell debris.
   • Transfer the lysate to a microcentrifuge tube.
   • Centrifuge the lysate at ~14,000 × *g* for 5 – 10 min. The soluble proteins are separated from the insoluble fraction and the cell debris during centrifugation.
4. Transfer the supernatant containing soluble protein to a new reaction tube and proceed with further analysis.

Table 1: Suggested volumes of Lysis-M Reagent containing c*O*mplete, EDTA-free tablet to use for different sizes of standard culture plates**.**

Plate Size/Surface Area	Volume of Lysis-M Reagent + c*O*mplete
100 mm[1)]	500 – 1,000 µl
60 mm	250 – 500 µl
6-well plate	200 – 400 µl per well
24-well plate	100 – 200 µl per well
96-well plate	50 – 100 µl per well

[1)] Cells grown in 100 mm plates typically contain 10^7 cells (50 mg). The typical yield resulting from the extraction of 10^7 cells is approx. 3 mg of total protein.

2.3 Protocol for Lysis of Mammalian Cells in Suspension

1 • Collect cells by centrifugation at 2,500 × *g* for 10 min.
• Decant the supernatant.
Optional: Wash cells once in washing buffer (*e.g.* PBS*) if the culture medium contains reagents that could interfere with subsequent protein analysis. Centrifuge the cells at 2,500 × *g* for 10 min after washing.

2 • Add at least 1 ml of Lysis-M Reagent containing c*O*mplete, EDTA-free tablet for each 100 mg (~100 µl) of wet cell pellet.
First, add 1/10 of the final recommended volume of Lysis-M Reagent containing c*O*mplete, EDTA-free tablet to the cells if large amounts of cells are used. Resuspend the pellet by pipetting up and down. Then add the rest of the Lysis-M Reagent containing c*O*mplete, EDTA-free tablet to the cell suspension.
Expect to obtain approximately 6 mg of total protein from100 mg of wet cell pellet depending on cell type.

3 Incubate the lysate for 10 min with gentle shaking.
Pellet cell debris by centrifugation at ~14,000 × *g* for 15 min.

4 Transfer the supernatant containing soluble protein to a new reaction tube and proceed with further analysis.

3. Typical Result

Cos-7 cells at confluency were harvested in Lysis-M Reagent containing c*O*mplete tablet. The extracted proteins were analysed by SDS-PAGE (5 µl/lane).
M: marker
W: whole protein fraction
S: supernatant fraction
P: pellet fraction

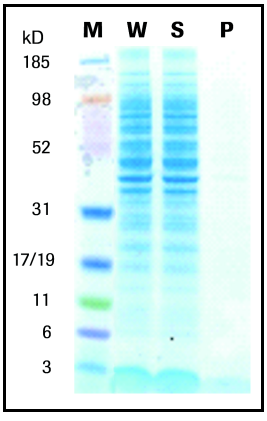

Fig. 1: SDS-PAGE analysis and Coomassie blue staining of proteins extracted from Cos-7 cells.

When directly dissolved in Lysis-M Reagent, the c*O*mplete, Mini, EDTA-free Protease Inhibitor Cocktail maintained full functionality for efficient inhibition of a multitude of proteases (including serine- and cysteine proteases but no metalloproteases).

Typical values for the inhibition of different proteases and protease mixtures by c*O*mplete, Mini, EDTA-free tablet in Lysis-M Reagent are shown in table 2.

Table 2: Inhibition of different proteases by c*O*mplete Protease Inhibitor Tablets.

Protease or protease mixture	Enzyme concentration (µg/ml)	% inhibition after immediate addition to the protease
Pancreatic extract	20	91%
Chymotrypsin	2.0	100%
Trypsin	0.2	92%
Papain	20	73%

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

** available from Roche Diagnostics*

4. Troubleshooting

Observation	Possible Cause	Recommendation
Low protein yield	Protein expression is low	Optimize the transfection procedure.
	Insufficient amounts of Lysis-M Reagent	Add more Lysis-M Reagent.
	Lysis-M Reagent was unable to penetrate the cell membrane	Increase incubation time and shake more vigorously during incubation.

5. Additional Information on this Product

Product Description

The c*O*mplete Lysis-M , EDTA-free protein extraction reagent for mammalian cells contains a mild detergent in 25 mM bicine buffer (pH 7.6). This simple extraction method allows efficient and gentle extraction of proteins from both the cytoplasm and the nucleus of cultured mammalian cells. Efficient lysis of mammalian cells occurs in only 5 minutes at +15 to +25°C eliminating the need for scraping, sonication or freeze-thaw cycles. The protein yields obtained with this kit are 20 to 25% higher compared to three cycles of freeze-thaw and approximately 20% higher than 2 minutes of sonication (with 50% pulse).

Proteases are ubiquitous in all living cells. As soon as cells are disrupted, proteases are released and can quickly degrade any protein (1). This can drastically reduce the yield of protein during isolation and purification. The c*O*mplete, Mini, EDTA-free tablets provided with this kit allow the inhibition of a broad spectrum of serine and cycsteine proteases, but not metalloproteases. In contrast to other c*O*mplete tablets they do not contain EDTA, thus leaving the stability and the function of metal-dependent proteins unaffected. The affinity purification of Poly-Histidine tagged fusionproteins via IMAC (immobilized metal affinity chromatography) is also facilitated (no dialysis necessary).

Due to the optimized composition of the tablets they show excellent protease-inhibiting effects and are therefore well suited for the protection of proteins isolated from mammalian cells. c*O*mplete, Mini, EDTA-free tablets contains both irreversible and reversible protease inhibitors. Metalloproteases and aspartic proteases are not inhibited. A significant advantage is that the protease inhibitor tablets can be directly dissolved in the Lysis-M protein extraction reagent of the kit. The extracted proteins can be further purified or analyzed in downstream applications. c*O*mplete, Mini, EDTA-free 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-M Reagent.

References

1 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 c*O*mplete, Mini, EDTA-free tablets 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 94% after one hour (detection with Universal Protease Substrate, casein, resorufin-labeled*).

The efficiency of cell lysis using Lysis-M 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 document:

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 Diagnostics.

Symbols

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

Symbol	Description
	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 document the following abbreviations are used:

Abbreviation	Meaning
f.c.	final concentration
PAGE	polyacrylamide gel electrophoresis

6.2 Changes to Previous Version

Editorial changes.

6.3 Ordering Information

	Product	Pack Size	Cat. No.
Complete Lysis	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
c*O*mplete Protease Inhibitor Cocktail Tablets in EASYpacks	c*O*mplete	20 tablets in foil blisters (for 50 ml each)	04 693 116 001
	c*O*mplete, Mini	30 tablets in foil blisters (for 10 ml each)	04 693 124 001
	c*O*mplete, EDTA-free	20 tablets in foil blisters (for 50 ml each)	04 693 132 001
	c*O*mplete, Mini, EDTA- free	30 tablets in foil blisters (for 10 ml each)	04 693 159 001
c*O*mplete Protease Inhibitor Cocktail Tablets in glass vials	c*O*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
	c*O*mplete, Mini	25 tablets in a glass vial (for 10 ml each)	11 836 153 001
	c*O*mplete, EDTA-free	20 tablets in a glass vial (for 50 ml each)	11 873 580 001
	c*O*mplete, Mini, EDTA- free	25 tablets in a glass vial (for 10 ml each)	11 836 170 001
Kits and Sets	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
	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
Individual Protease Inhibitors	Aprotinin	10 mg 50 mg 100 mg	10 236 624 001 10 981 532 001 11 583 794 001
	Bestatin	10 mg	10 874 515 001
	Calpain Inhibitor I	25 mg	11 086 090 001
	Chymostatin	10 mg	11 004 638 001
	E-64	10 mg 25 mg	10 874 523 001 11 585 681 001
	Leupeptin	5 mg 25 mg 50 mg 100 mg	11 017 101 001 11 017 128 001 11 034 626 001 11 529 048 001
	α_2-Macroglobulin	25 inhibitory units	10 602 442 001
	Pefabloc® SC	100 mg 500 mg 1 g	11 429 868 001 11 585 916 001 11 429 876 001
	Pepstatin	2 mg 10 mg 50 mg	10 253 286 001 11 359 053 001 11 524 488 001
	PMSF	10 g 25 g	10 837 091 001 11 359 061 001
	TLCK – HCl	100 mg	10 874 485 001
	Trypsin Inhibitor (chicken, egg white)	1 g	10 109 878 001
	Trypsin Inhibitor (soybean)	50 mg	10 109 886 001
Buffers	Buffers in a Box, Premixed PBS Buffer, 10×	4 l	11 666 789 001

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