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Not for use in diagnostic procedures.

cOmplete Lysis-M EDTA-free

Version: 05
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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 1 kit
lysis of up to 100 g of mammalian cells

Store the product at +15 to +25°C.

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1. General Information

1.1. Contents

Label	Function / Description	Content
cOmplete Lysis-M, EDTA-free, Lysis-M Reagent	Contains a mild detergent in 25 mM bicine buffer, pH 7.6.	1 bottle, 200 ml
cOmplete, Mini, EDTA-free, Protease inhibitor cocktail tablets	▪ Individually packed tablets. ▪ Each tablet is sufficient for a volume of 10 ml solution.	20 foil blister packs, 1 tablet each

1.2. Storage and Stability

Storage Conditions (Product)

When stored at +15 to +25°C, the product is stable through the expiry date printed on the label.

Vial / bottle	Label	Storage
1	Lysis-M Reagent	Store at +15 to +25°C.
2	cOmplete, Mini, EDTA-free, Protease inhibitor cocktail tablets	

1.3. Additional Equipment and Reagent required

For cell washing

- PBS*

1.4. 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 minutes at +15 to +25°C, eliminating the need for scraping, sonication, or freeze-thaw cycles. The protein yields obtained are 20 to 25% higher compared to three cycles of freeze-thaw and approximately 20% higher than 2 minutes of sonication (with 50% pulse).
Lysis-M Reagent is compatible with different applications:

- Reporter assays, such as β-galactosidase, luciferase, and chloramphenicol acetyltransferase.
- Immunoassays, such as western blots, ELISAs, and RIAs.
- Protein assays, such as protein kinase A, protein kinase C, and tyrosine kinase.
- Protein purification

The cell lysate is compatible with protein assays, such as Coomassie staining and BCA (bicinchoninic acid) protein assays.

The reagent can be removed by dialysis.

2. How to Use this Product

2.1. Before you Begin

General Considerations

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 cOmplete, Mini, EDTA-free tablets provided with this kit allow the inhibition of a broad spectrum of serine and cysteine.
- Due to the optimized composition of the tablets, they show excellent protease-inhibiting effects and are very well suited for the protection of proteins isolated from mammalian cells.
- cOmplete, Mini tablets contains both irreversible and reversible protease inhibitors; the protease inhibitor tablets can be directly dissolved in the Lysis-M protein extraction reagent of the kit.

⚠ ***cOmplete, Mini, EDTA-free tablets are used 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 and aspartic proteases.***

Safety Information

Do not eat the tablets.

Laboratory procedures

- Handle all samples as if potentially infectious, using safe laboratory procedures. As the sensitivity and titer of potential pathogens in the sample material varies, the operator must optimize pathogen inactivation by the Lysis / Binding Buffer or take appropriate measures, according to local safety regulations.
- Do not eat, drink or smoke in the laboratory work area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats and eye protection, when handling samples and kit reagents.
- Wash hands thoroughly after handling samples and reagents.

Waste handling

- Discard unused reagents and waste in accordance with country, federal, state, and local regulations.
- Safety Data Sheets (SDS) are available online on dialog.roche.com, or upon request from the local Roche office.

Working Solution

One cOmplete, Mini, EDTA-free Protease inhibitor tablet is sufficient for the inhibition of the proteolytic activity in 10 ml Lysis-M Reagent.

1. Add one cOmplete, Mini, EDTA-free tablet in 10 ml Lysis-M Reagent.

2. Incubate for 2 minutes at +15 to +25°C.

3. Vortex briefly.

2.2. Protocols

Lysis of adherent mammalian cells

For adherent mammalian cells, the maximum cell lysis without cell scraping can be obtained by using the volumes of Lysis-M Reagent specified below. If necessary, estimate the volume of cells to calculate the required volume of Lysis-M Reagent. For example, 2 × 10^6 of HeLa cells is equivalent to 20 mg of cells, approximately 10 µl of a packed cell volume, and requires 200 µl of Lysis-M Reagent. For more concentrated cell extracts, use a smaller volume of Lysis-M Reagent. In this case, the cells must be scraped for maximum recovery.

1. Remove (decant) culture media from the adherent cells grown in monolayer culture.
   – Optional: Wash cells once in a washing buffer, such as 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, Mini, EDTA-free tablets to each plate or well as shown in the table.

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

   – Incubate for 5 minutes at +15 to +25°C 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 approximately 14,000 × *g* for 5 to 10 minutes to separate the soluble proteins from the insoluble fraction and the cell debris.

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

(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 approximately 3 mg of total protein.

Lysis of mammalian suspension cells

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

2. Add at least 1 ml of Lysis-M Reagent containing cOmplete, Mini, EDTA-free tablets for each 100 mg, approximately 100 µl of wet cell pellet.
   If large amounts of cells are used, initially add only 1/10 of the recommended volume of Lysis-M Reagent containing cOmplete, Mini, EDTA-free tablets. Resuspend the pellet by pipetting up and down, then add the rest of the Lysis-M Reagent containing cOmplete, Mini EDTA-free tablets to the cell suspension.
   Expect to obtain approximately 6 mg of total protein from 100 mg of wet cell pellet, depending on cell type.

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

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

3. Results

Typical result

Cos-7 cells at confluency were harvested in Lysis-M Reagent containing cOmplete, Mini tablets. The extracted proteins were analyzed by SDS-PAGE (5 µl/lane).
M: Marker
W: Whole protein fraction
S: Supernatant fraction
P: Pellet fraction

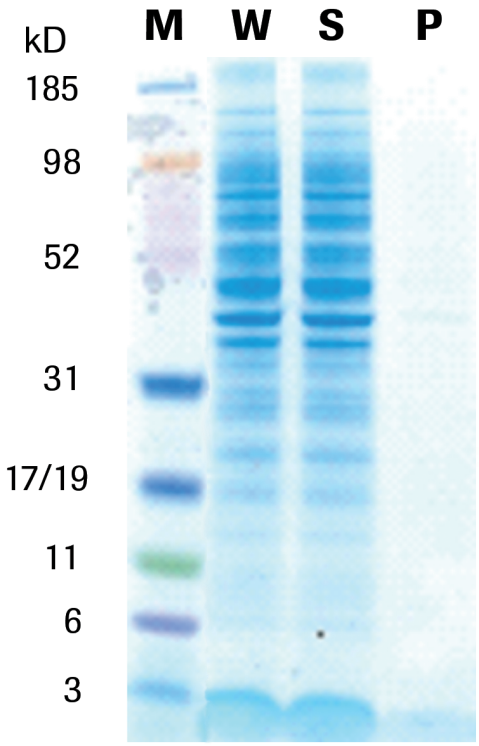

Fig. 1: SDS-PAGE analysis and Coomassie blue staining of proteins extracted from Cos-7 cells.

Inhibition of different proteases

One cOmplete, Mini, EDTA-free tablet was added per 10 ml Lysis-M Reagent. Proteolytic activity was determined with Universal Protease Substrate* (casein, resorufin-labeled). For extractions or single-step isolations in the acid pH range, include Pepstatin* along with cOmplete, Mini, EDTA-free tablets to ensure aspartic (acid) protease inhibition. All experiments were performed at +15 to +25°C.

When directly dissolved in Lysis-M Reagent, the cOmplete, Mini, EDTA-free Protease inhibitor cocktail maintained full functionality for efficient inhibition of a multitude of proteases (including serine- and cysteine proteases, but not metalloproteases). Typical values for the inhibition of different proteases and protease mixtures is shown in the table.

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

4. Troubleshooting

Observation	Possible cause	Recommendation
Low protein yield.	Protein expression is low.	Optimize 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

5.1. Quality Control

For lot-specific certificates of analysis, see section **Contact and Support**.

6. Supplementary Information

6.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

6.2. Changes to previous version

Layout changes.
Editorial changes.
Update to include new safety Information to ensure handling according controlled conditions.

6.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
Buffers in a Box, Premixed PBS Buffer, 10x	4 l	11 666 789 001

6.4. Trademarks

All product names and trademarks are the property of their respective owners.

6.5. License Disclaimer

For patent license limitations for individual products please refer to:
List of biochemical reagent products.

6.6. Regulatory Disclaimer

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

6.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

6.8. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

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Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim
Germany