



3050 Spruce Street  
Saint Louis, Missouri 63103 USA  
Telephone (800) 325-5832 (314) 771-5765  
Fax (314) 286-7828  
email: techserv@sial.com  
sigma-aldrich.com

## ProductInformation

### CellLytic™ Y

Yeast Cell Lysis/Extraction Reagent

Product Number **C 4482**

Store at Room Temperature

## TECHNICAL BULLETIN

### Product Description

Yeast cells are difficult to lyse due to their rigid cell wall. Commonly used methods for yeast protein extraction, involve the inconvenient use of mechanical force (glass beads at 4 °C), enzymes or extreme conditions, which could result in protein inactivation (pH, temperature, and chloroform).

The CellLytic™ Y reagent offers a convenient method for efficient cell lysis and protein solubilization, while avoiding protein degradation and interference with protein immunoreactivity and biological activity.

Use of CellLytic Y reagent involves a short procedure (20 – 30 minutes) performed at room temperature using standard lysis conditions (i.e. mild detergent and pH 8) without the need for extreme conditions or glass beads required by other methods.

CellLytic Y can be used for extraction of proteins from fresh or frozen yeast cells. The cell extract is compatible with:

- Reporter gene expression assays (for example,  $\beta$ -galactosidase, alkaline phosphatase)
- Immunoassays (Western blots, Immunoprecipitation)
- Affinity based purification (FLAG, glutathione S- transferase (GST), and histidine-tagged fusion proteins)
- DNA-protein interaction assay (gel-shift)
- Coomassie® blue and silver staining of gels
- Assays in which phosphate participates in the reaction (i.e. alkaline and general phosphatase assays)

For some applications, performing the procedure at 4 °C and/or the addition of specific components may be advantageous. Examples for components to be added: protease or phosphatase inhibitor cocktail, reducing agents, chelators, or different salts.

The efficiency of CellLytic Y reagent for protein extraction has been tested on different strains of *Saccharomyces cerevisia* [Y187, Y190, W303(a), S288C, SP1,  $\Sigma$ 1278b (diploid and haploid), BJ2168, and cdc25] and *Schizosaccharomyces pombe*.

### Reagents Provided

Sufficient CellLytic Y reagent is provided for extraction of 100 – 200 grams of packed yeast cells.

### Reagents and Equipment Required but Not Provided

(Product numbers are given where available)

- Protease inhibitor cocktail, Product No. P8215
- Dithiothreitol (DTT), Product No. D9779
- Test tubes
- Shaker
- Centrifuge
- Microcentrifuge Eppendorf® 5417R (Product No. Z366013 or Z366021) or equivalent

### Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

### Storage/Stability

Store the product at room temperature.

### Procedure

For best cell lysis and protein extraction, cells should be harvested during log-phase growth.

In general, it is recommended to add protease inhibitor cocktail.

Addition of glass beads may increase yield of extraction of high molecular weight proteins (>70 kDa) or when the culture is past log-phase growth.

For best results when using *Schizosaccharomyces pombe*, the cells should be grown in a synthetic defined medium (Edinburgh Minimal Medium, EMM). If cells are grown in a rich medium (YES), they must be harvested during log-phase growth. If cell growth conditions are not optimal, the addition of glass-beads may increase yield.

1. Collect the cells in an appropriate centrifuge conical test tube. Centrifuge for 5 minutes at  $\sim 3,000 \times g$  at 4 °C. Remove the supernatant and discard.
2. For all cases in which the addition of DTT does not interfere with the application tested, it is recommended to add DTT (5 – 10 mM final concentration) to the CellLytic Y reagent prior to step 3. Addition of DTT significantly improves total protein yield.
3. Resuspend the cell pellet in the appropriate volume of CellLytic Y reagent. It is recommended to add 2.5 – 5 ml of CellLytic Y reagent per 1 gram of yeast cell pellet. The volume of CellLytic Y reagent to be added to the cells varies directly with the mass of wet cell pellet and depends on the protein concentration required.
4. Shake the cells gently for 15 – 30 minutes.
5. Centrifuge the lysed cells for 10 minutes at 12,000 – 20,000  $\times g$  to pellet the cellular debris.
6. Transfer the protein-containing supernatant to a chilled test tube. For immediate use, keep on ice. Otherwise, store the protein solution at –20 °C (or at –70 °C for improved stability).

#### Related Products

- CellLytic B, Bacterial Cell Lysis/Extraction Reagent, Product No. B7435
- CellLytic B 2x, Bacterial Cell Lysis/Extraction Reagent, Product No. B7310
- CellLytic M, Mammalian Cell Lysis/Extraction Reagent, Product No. C2978
- CellLytic MT, Mammalian Tissue Lysis/Extraction Reagent, Product No. C3228
- Mammalian Cell Lysis Kit, Product No. MCL1
- CellLytic NuCLEAR Extraction Kit, Product No. NXTRACT

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## Troubleshooting Guide

| Problem                                                              | Cause                                                                   | Solution                                                                                                                                                                                                                                                                                                                                      |
|----------------------------------------------------------------------|-------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Low yield of total protein                                           | Culture was not grown from a fresh starter or was grown past log phase. | Use a fresh starter.                                                                                                                                                                                                                                                                                                                          |
|                                                                      |                                                                         | Grow culture to log phase.                                                                                                                                                                                                                                                                                                                    |
|                                                                      |                                                                         | When culture is significantly past log phase or not fresh, following step 3 add 4 grams of glass beads (G8772) per gram of wet cell pellet. Instead of the shaking step, vortex the suspension at maximal speed for 30 seconds at 4 °C. Repeat this step 5 - 6 times. After each vortex incubate the suspension for 1 minute on ice.          |
| Low yield of protein extracted from <i>Schizosaccharomyces pombe</i> | Non-optimal growth conditions                                           | For best results when using <i>Schizosaccharomyces pombe</i> , the cells should be grown in a synthetic defined medium (Edinburgh Minimal Medium, EMM). If cells are grown in a rich medium (YES), they must be harvested during log-phase growth. If cell growth conditions are not optimal, the addition of glass beads may increase yield. |
| Low yield of high molecular weight (>70 kDa) proteins                | Non-optimal growth conditions                                           | To increase high-molecular weight protein yield, following step 3 add 4 grams of glass beads (G8772) per gram of wet cell pellet. Instead of the shaking step, vortex the suspension on maximal speed for 30 seconds at 4 °C. Repeat this step for 5 - 6 times. After each vortex incubate the suspension for 1 min on ice.                   |
| Protein degradation                                                  | Active proteases in the extract                                         | Add Protease Inhibitor Cocktail (P8215)                                                                                                                                                                                                                                                                                                       |

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