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

CelLytic™ Y Plus
For Enzymatic Lysis of Yeast

Catalog Number **CYP1**

TECHNICAL BULLETIN

Product Description

Sigma's Yeast Enzymatic Lysis Kit provides a convenient method for highly efficient spheroplast formation and protein extraction from *Saccharomyces cerevisiae*, *Pichia pastoris*, and *Schizosaccharomyces pombe* yeast strains. The procedure involves spheroplast formation by enzymatic digestion of the yeast cell wall followed by cell protein extraction (with or without detergent), while avoiding protein degradation and reagent interference with protein immunoreactivity and biological activity.

Cells extracts are compatible with:

- Reporter gene expression assays (β-galactosidase or alkaline phosphatase)
- Immunoassays (Western blots or immuno-precipitation assays)
- Affinity based purification [FLAG®, glutathione S-transferase (GST), and histidine-tagged fusion proteins].
  Note: The Protease Inhibitor Cocktail provided in the kit is not compatible with histidine-tagged protein purification. For this application use Protease Inhibitor Cocktail for histidine tagged proteins, Catalog Number P8849.
- DNA-protein interaction assay (gel-shift)
- Coomassie® Blue and silver staining of gels

Components

Sufficient reagents are provide for spheroplast formation and protein extraction from 10–40 grams of yeast cell pellet.

Reaction Buffer (Catalog Number R4902)	120 ml
1 M DTT (Catalog Number D7059)	5 ml
Lyticase (1 ml solution, 25 units/µl) (Catalog Number E6904)	25,000 units
Extraction Buffer (Catalog Number E6529)	60 ml
10% TRITON® X-100 (Catalog Number T5444)	10 ml
Protease Inhibitor Cocktail (Catalog Number P8215)	2 ml

Equipment Required For Extraction but Not Provided

- Test tubes
- Centrifuge (Sorvall® with SS-34 rotor or equivalent)
- Incubator
- Homogenizer (optional)
- Spectrophotometer or Microscope

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

The kit is shipped on dry ice. The DTT solution, Protease Inhibitor Cocktail, and Lyticase enzyme should be stored at –20 °C. The other kit components can be stored at 2–8 °C. After the first thaw of the DTT solution, it is recommended to aliquot the solution and store it at –20 °C.

Procedures
Be sure all solutions are mixed thoroughly before use. Remove aliquots from the kit components in an aseptic manner. The quantities given in the procedure are for protein extraction from 2 x 10^9 *Saccharomyces cerevisiae* cells (180–200 mg). For different amounts, calculate accordingly

A. Spheroplast Preparation
Spheroplast preparation should be monitored closely and the enzymatic reaction stopped as soon as spheroplasting occurs. Longer digestion times may have deleterious effects on subsequent procedures. Refer to Spheroplasts Lysis Monitoring (section B) for methods of monitoring the degree of conversion to spheroplasts.

1. Pellet yeast cells (2 x 10^9 cells) from mid-log phase (1-5 x 10^7 cells/ml) by centrifugation at 3,000–5,000 x *g* for 5 minutes.
2. Resuspend the pellet with 4–6 volumes of distilled water (5 ml) and transfer to preweighed tube.
3. Centrifuge at 3,000–5,000 x *g* for 5 minutes and weigh the pellet in order to determine the amount of lyticase required for efficient spheroplasting.
4. Resuspend the pellet in 1–2 volumes (300 µl) of Reaction Buffer containing DTT at a final concentration of 30 mM (i.e., addition of 30 µl of 1 M DTT to 970 µl Reaction Buffer).
   Note: The DTT should be freshly added to each reaction mixture. Remove a sample for Optical Density Measurement (see section B).
5. Transfer the suspended cells to a microcentrifuge tube.
6. Add Lyticase (4–5 µl). Each strain of yeast requires a different concentration of enzyme for efficient lysis. For optimal concentration, suitable for the different strains, see Table 1.

Table 1.

Yeast	Units lyticase/ g cells	Reaction Buffer Volume	Reaction Time (min) at 37 °C
S. cerevisiae	625 (25 µl)	1-2× packed cell volume	15-30
P. pastoris	625 (25 µl)	1× packed cell volume	15-30
S. pombe	2,500 (100 µl)	1× packed cell volume	30-45

7. Incubate the reaction tube at 37 °C for 15–30 minutes (for *S. pombe* 30–45 minutes). Monitor the spheroplasting reaction according to Spheroplasts Lysis Monitoring (section B) and finalize the incubation.
8. Pellet the spheroplasts by centrifugation at 1,500 x *g* for 5 minutes.
9. The spheroplasts at this stage can be stored at –20 °C or can be used directly for protein extraction.

B. Spheroplasts Lysis Monitoring
The yeast cell lysis can be monitored to indicate spheroplast formation by two assays:

1. Observing spheroplast formation under the microscope.
2. Measuring the optical density of a reaction sample at 800 nm. Before the addition of the lyticase enzyme (pre-lysed sample, section A, step 4), place a 10 µl sample from the reaction mixture into 990 µl water and read the OD at 800 nm. After the addition of the lyticase enzyme (during the incubation of the sample at 37 °C, section A, step 7), every 10–15 minutes, take a 10 µl sample, add it to 990 µl water and read the OD at 800 nm. When the OD is 10% to 20% of the pre-lysed sample reading, end the incubation.

C. Protein Extraction

Spheroplast disruption can be performed using physical force or a detergent. The use of physical force is recommended for cases in which the detergent will interfere with further analyses. Otherwise, using a detergent simplifies the procedure and results in a higher protein yield.

For inhibition of proteases, it is recommended to add protease inhibitor cocktail during the extraction procedure. In general, 1 ml is recommended for the inhibition of proteases in 20 g of yeast. For 500 µl of Extraction Buffer (used for protein extraction from 2×10^9 cells), add 10 µl of the Protease Inhibitor Cocktail. The Protease Inhibitor Cocktail provided in the kit is not compatible with histidine-tagged protein purification. For this application use Protease Inhibitor Cocktail for histidine tagged proteins, Catalog Number P8849.

Some proteins are better extracted when DTT or EDTA is present in the Extraction buffer. For this purpose, add 2 mM DTT and/or 2 mM EDTA (final concentration) to the Extraction Buffer before mixing with the spheroplasts.

1. a. For spheroplast disruption using a detergent – Resuspend the spheroplast pellet with 2–3 volumes (500 µl) of Extraction Buffer containing 1% TRITON X-100 (i.e. add 50 µl of 10% TRITON X-100 to 450 µl of Extraction Buffer). Pipette up and down and vortex.

   b. For protein extraction using physical force – Resuspend the spheroplast pellet with 2–3 volumes of Extraction Buffer. Pipette up and down, and then vortex. Homogenize the spheroplasts in suspension with an appropriate homogenizer.
2. Incubate the suspension for 15–30 minutes at room temperature, while shaking.
3. Centrifuge at 12,000 x *g* for 15 minutes.
4. Transfer the supernatant to a clean chilled test tube. For storage, snap-freeze aliquots of the supernatant in liquid nitrogen and store at –70 °C.

EB,CMH,MAM 11/06-1