# Mammalian Protein Extraction Buffer Yeast Protein Extraction Buffer Kit

Mammalian Protein Extraction Buffer and Yeast Protein Extraction Buffer Kit offer a convenient method to extract total soluble protein from mammalian cultured cells and yeast cells, respectively (Fig 1). In the past, physical methods of cell disruption were commonly used which were laborious, vigorous, and often required expensive equipment. The Mammalian Protein Extraction Buffer and Yeast Extraction Buffer Kit are mild, detergent-based cell lysis methods, eliminating the need for mechanical cell lysis, and delivering high quality protein lysate compatible with most downstream applications.

**Fig 1.** The Mammalian Protein Extraction Buffer and Yeast Protein Extraction Buffer Kit allow for efficient and convenient protein extractions.

### Key benefits include:

- High protein recovery – stable formulations give efficient protein extraction
- Maintained biological activity – mild, non-denaturing buffer compositions
- High reproducibility – high quality reagents produce consistent results
- High compatibility – compatible with most downstream applications
- Convenience – ready to use buffers save time by eliminating the need for homemade buffers. The Yeast Protein Extraction Buffer Kit allows for efficient extraction without having to use glass beads.
- Transparency – full declaration of buffer components

## Description

Mammalian Protein Extraction Buffer is a 500 ml solution designed for efficient and gentle extraction of total soluble proteins from mammalian cultured cells. This buffer can be used both for cell suspensions and adherent cells. Yeast Protein Extraction Buffer Kit is developed for mild extraction of soluble proteins from yeast cells. This kit eliminates the need for glass beads, a common method of mechanical lysis for yeast cells, and is a proprietary improvement on the Zymolyase™ based method for spheroplast preparation and extraction of soluble proteins from yeast cells. Both extraction buffers address the need for simple and versatile sample preparation methods for protein extraction.

The Mammalian Protein Extraction Buffer and Yeast Protein Extraction Buffer Kit are based on organic buffering agents, mild nonionic detergents, and a combination of various salts and agents to gently disrupt the cell wall and release soluble proteins (Table 1). Depending on the application, additional agents such as chelating agents and reducing agents may be added directly to the Mammalian Protein Extraction Buffer and Yeast Protein Extraction Buffer Kit before use. If protease inhibition is required, a cocktail of protease inhibitors may be added to prevent protein degradation (see related products, Protease Inhibitor Mix).

The Yeast Protein Extraction Buffer Kit is suitable for preparation of approximately 10 ml yeast cell pellet suspension, either as a single preparation or as multiple, smaller preps. A ready to use Zymolyase preparation and Yeast Suspension Buffer are also provided in the kit.

**Table 1.** Product characteristics

| Mammalian Protein Extraction Buffer | Contains |
|---|---|
| 500 ml | < 20mM Tris hydroxymethyl-aminomethane |
| | < 20mM sodium chloride |
| | < 5% NP-40* |
| | < 5% Triton X-100† |
| | < 5% Tween 20‡ |
| **Yeast Protein Extraction Buffer Kit** | **Contains** |
| Yeast Protein Extraction Buffer, 100 ml | < 100mM Tris hydroxymethyl-aminomethane |
| | < 100mM sodium chloride |
| | < 5% NP-40* |
| | < 5% Triton X-100† |
| | < 5% Tween 20‡ |
| Yeast Suspension Buffer, 15 ml | <10% sodium chloride |
| | < 1% potassium chloride |
| | < 5% sodium phosphate dibasic anhydrous |
| | < 1% potassium phosphate, monobasic |
| *Longlife*™ Zymolyase, 2 x 0.5 ml | Lyticase, stabilized preparation (1500 U/ml) |
| | ammonium sulfate |

* Nonylphenyl polyethylene glycol

† Octylphenolpoly(ethyleneglycolether

‡ Polyethylene glycol sorbitan monolaurate

The Yeast Suspension Buffer is used during the cell lysis step, together with Zymolyase, an enzyme that digests the cell wall layer of yeast. After cell lysis, Yeast Protein Extraction Buffer is added to the yeast pellet, referred to as spheroplast, to extract biologically active, soluble protein.

## Compatibility

Mammalian Protein Extraction Buffer is compatible with most applications, including chromatography procedures, electrophoresis, and enzyme assays such as reporter gene assays (e.g., ß-galactosidase, luciferase, chloramphenical acetyltransferase), kinase assays (e.g., PKC, PKA, Tyrosine Kinase), and immunoassays (e.g., ELISA, Western blots, RIA).

Yeast Protein Extraction Buffer Kit is compatible with any downstream application including enzyme assays, chromatography procedures, and gel electrophoresis applications. The Yeast Protein Extraction Buffer Kit is provided with a supplementary protocol for making spheroplast and for removing the lytic enzyme Zymolyase, prior to lysis and extraction of yeast proteins.

## Efficient and reproducible protein extraction

Mammalian Extraction Buffer gives efficient protein extraction with high yield and reproducibility. In Figure 2A, protein lysate, extracted from Chinese hamster ovary (CHO) cells using Mammalian Protein Extraction Buffer, demonstrated high yield and reproducibility. In Figure 2B, ten replicates of protein extracts from *Saccharomyces cerevisiae* gave high reproducibility using Yeast Protein Extraction Buffer Kit. In a study preparing protein extracts from two different yeast strains, *S. cerevisiae* and *Pichia pastoris*, high yield was attained for both strains using the Yeast Protein Extraction Buffer Kit (Fig 3).

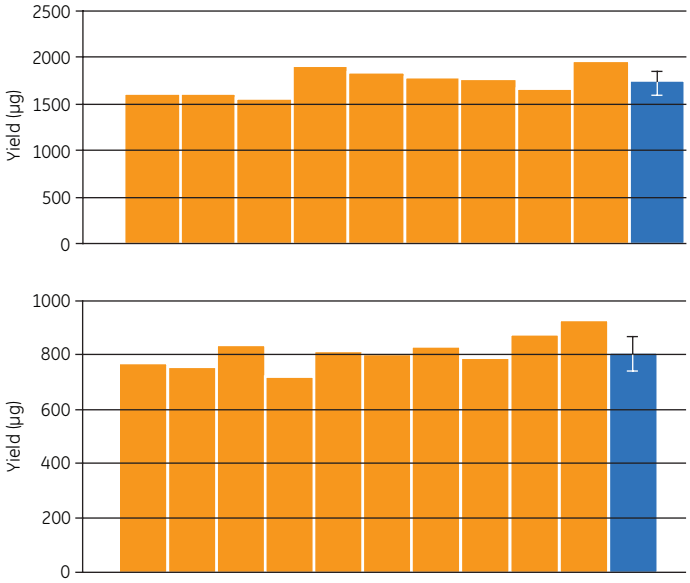


**Fig 2.** High reproducibility using A) Mammalian Protein Extraction Buffer and B) Yeast Protein Extraction Buffer Kit. Total yield for individual samples is shown, as well as the average and standard deviation. In the extraction using Mammalian Protein Extraction Buffer, 0.3 ml was used to lyse and extract ten samples containing $1x10^7$ CHO cells. One sample is not shown due to bubbles in the well. In the extraction using Yeast Protein Extraction Buffer Kit, 0.1 ml was used to extract ten samples containing 50 mg of *S. cerevisiae*.

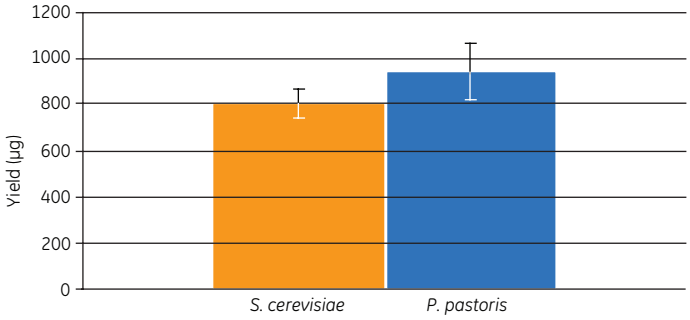


**Fig 3.** High protein yield from different yeast strains. Total yield after protein extraction was measured using the 2-D Quant Kit. 50 mg of yeast paste (*S. cerevisiae* or *P. pastoris*) was lysed in 0.4 ml Yeast Protein Extraction Buffer.

## Retained protein activity

To evaluate retained protein activity, the CHO cell extracts (Fig 2A) were assayed using a carbonic anhydrase activity assay. The relative carbonic anhydrase activity for each sample was corrected for sample concentration, and the average is presented in Figure 4. As a control, protein extracts from CHO cells were prepared using homemade RIPA buffer, and the result from the carbonic anhydrase activity assay is shown (Fig 4). The figure shows that protein activity is retained using the Mammalian Protein Extraction Buffer, and the results were comparable to using homemade extraction buffer.

Mechanical cell disruption with glass beads is a common method for lysing yeast cells. Homemade RIPA buffer, together with glass beads, was used to lyse and extract protein from *S. cerevisiae* or *P. pastoris*. These samples, as well as the protein extracts prepared using the Yeast Protein Extraction Buffer Kit (Fig 2B), were measured for retained protein activity using an alkaline phosphatase assay. This assay measures the conversion of the substrate pNPP by alkaline phosphatase over time. The relative activity was corrected for sample concentration, and the averages are shown in Figure 5. For both yeast strains, the retained protein activity using the Yeast Protein Extraction Buffer Kit gave consistent results that were comparable to the conventional method using glass beads.

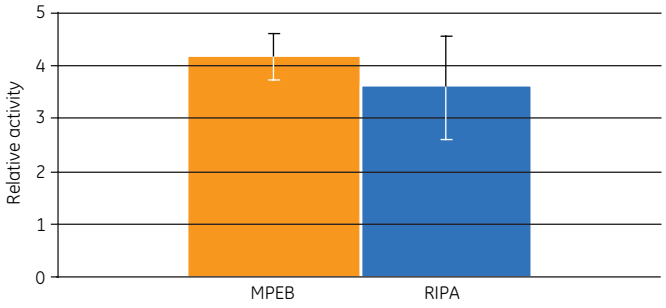


**Fig 4.** Carbonic anhydrase activity in CHO cell extracts prepared by Mammalian Protein Extraction Buffer (MPEB) or homemade RIPA buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 1% NP-40, and 0.1% Na-deoxycholate). The relative activity, corrected for differing sample concentrations, was calculated and the average and standard deviation are shown. The figure shows that high protein activity was retained using both methods.

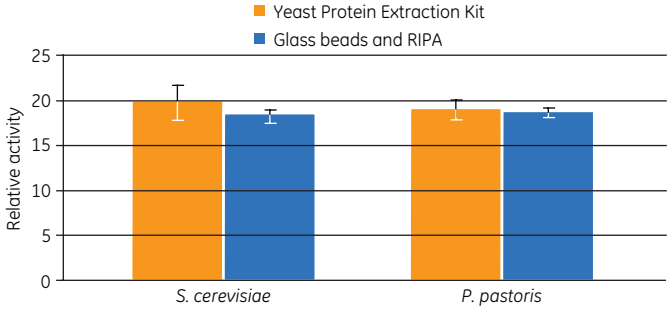


**Fig 5.** Alkaline phosphatase activity in yeast extracts prepared using either homemade RIPA buffer with glass beads or Yeast Protein Extraction Buffer. The relative activity, corrected for sample concentration, was calculated and the average and standard deviation are shown. High protein activity was retained using both methods.

## Storage

The products are shipped at ambient temperature. Upon arrival, the Mammalian Extraction Buffer and Yeast Extraction Buffer Kit should be stored at 4–8°C, and are stable for one year when stored and used as recommended. The lytic enzyme Zymolyase must be stored at –20°C.

## Ordering information

| Products | Quantity | Code no. |
|---|---|---|
| Mammalian Protein Extraction Buffer | 1 x 500 ml | 28-9412-79 |
| Yeast Protein Extraction Buffer Kit | 1 x kit (10 ml yeast cell pellet suspension) | 28-9440-45 |

*Related products*

| Products | Quantity | Code no. |
|---|---|---|
| 2-D Quant Kit | 500 assays | 80-6483-56 |
| Nuclease Mix | 0.5 ml | 80-6501-42 |
| Protease Inhibitor Mix | 1 ml | 80-6501-23 |
| SDS-PAGE Clean-Up Kit | | 80-6484-70 |
| His SpinTrap™ | 50 × columns | 28-4013-53 |
| His MultiTrap™ HP | 4 × 96-well plates | 28-4009-89 |
| His MultiTrap FF | 4 × 96-well plates | 28-4009-90 |
| His GraviTrap™ | 10 × 1 ml columns | 11-0033-99 |
| HisTrap™ FF crude | 5 × 1 ml | 11-0004-58 |
| GST SpinTrap Purification Module | 50 × columns, buffer kit | 27-4570-03 |
| GST MultiTrap FF | 4 × 96-well plate | 28-4055-01 |
| GST MultiTrap 4B | 4 × 96-well plate | 28-4055-00 |
| GSTrap™ 4B | 5 × 1 ml | 28-4017-45 |
| PD-10 Desalting Columns | 30 × columns | 17-0851-01 |
| Vivaspin™ ultracentrifugation devices | Multiple | |

| Related literature | Code no. |
|---|---|
| Recombinant Protein Purification Handbook, Principles and Methods | 18-1142-75 |
| Selection guide for protein and nucleic acid sample preparation | 28-9337-00 |

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