

P01013**Total protein extraction kit**

Product	The total protein extraction kit has been specially formulated to extract total protein from fungi such as <i>Saccharomyces cerevisiae</i> or <i>Pichia pastoris</i> . Its unique detergent mix ensures uniform extraction of all proteins from a cell, including integral membrane proteins and membrane-associated proteins.
Contents	Extraction Buffer H (20 mL) Sample Buffer (9 mL) Glass beads (12 mL) Sufficient for 60 sample preparations
Storage	Store at room temperature

Application Protein extraction from fungi and bacteria can be challenging, due to the stable cell wall which must be disrupted prior to protein extraction and solubilization. Most often, mechanical disruption methods, such as French Press or glass beads are used to remove the cell wall, followed by detergent based extraction of total protein.

The total protein extraction kit has been specially formulated for efficient extraction and solubilization of total protein mixtures from fungi and bacteria. After mechanical disruption of the cell wall by glass beads, proteins are solubilized using a complex detergent mix. The use of several detergents ensures uniform solubilization of proteins with widely different characteristics, including strongly hydrophobic proteins, such as integral membrane proteins. After solubilization, proteins are denatured by addition of sample buffer and loaded onto an SDS-PAGE gel for further analysis.

Protocol for preparation of total protein extracts from *Saccharomyces cerevisiae*

- 1 Add 30 mL appropriate SD medium to a 50 mL Falcon tube
- 2 Use a sterile loop to scrape yeast cells off the SD plate. Make sure to use fresh yeast (no older than 2-3 days) and use approx. one loop full of cells
- 3 Inoculate the liquid culture with the yeast cells
- 4 Close the Falcon tube and vortex for 1 minute to disperse the cells
- 5 Grow overnight at 30°C with shaking at 250 rpm to an OD₅₄₆ of 0.6-0.8
- 6 Precool the Extraction Buffer H on ice and supplement with appropriate protease inhibitors
- 7 Pellet the culture at 2500x g for 5 minutes
- 8 Add 300 µL ice cold Extraction Buffer H to each pellet and resuspend by vortexing
- 9 Transfer to eppendorf tubes

- 10 Add approx. 200 μL glass beads to each tube
- 11 Vortex samples 5 times for 30 seconds, with intermittent cooling on ice for 2 minutes
Note
The most efficient extraction is achieved by using a cell disruptor such as the FastPrep machine (ThermoSavant). If you have access to a cell disruptor, we strongly suggest using it. Usually, 3 runs of 45 seconds at maximum intensity with intermittent cooling on ice for 5 minutes is sufficient to ensure lysis of > 95% of all cells.
- 12 Incubate tubes on ice with shaking for 15 minutes
- 13 Centrifuge at 14'000 g for 20 minutes
- 14 Prepare the appropriate amount of supplemented Sample Buffer by adding 40 $\mu\text{L}/\text{mL}$ β -mercaptoethanol
Note
 β -mercaptoethanol should be handled under a fume hood. Wear appropriate protective clothing.
- 15 Carefully transfer 100 μL of the supernatant from Step 13 to a fresh tube
- 16 Mix the 100 μL supernatant with 50 μL supplemented Sample Buffer and keep at room temperature
- 17 Discard the remaining supernatant and resuspend the pellet in 100 μL supplemented Sample buffer by vigorous vortexing
- 18 Incubate the supernatant and pellet samples at 37°C for 15 minutes
- 19 Load 5-10 μL per sample on SDS-PAGE or store at -20°C

Support

Please see www.dualsystems.com for support and protocols. Please direct support inquiries to support@dualsystems.com or call +41 44 738 50 00.

Research use

This product is intended for research use only, not for diagnostic or therapeutic uses. Observe good laboratory practice guidelines and wear gloves, laboratory coat and glasses when handling this kit and its components.