PROMPT
Proteins for Research

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Fungal Protein Extraction Kit

Cat.NO: R0048

Introduction

Fungal Protein Extraction Kit is designed for efficient extraction of soluble proteins from fungal cells. The kit facilitates cell wall weakening and spheroplast formation, enabling easy cell lysis and improved protein release. A standardized protocol is provided to ensure consistent and reproducible results.

The Fungal Protein Extraction buffer is formulated with organic buffering agents, mild non-ionic detergents, and a proprietary combination of salts to enhance protein yield and stability. It is compatible with the addition of reducing agents, chelating agents, and protease inhibitors, depending on specific application requirements.

This kit provides a simple, reliable, and time-efficient method for Fungal Protein Extraction while eliminating the need for labor-intensive techniques such as glass bead lysis.

Kit Contents

Contents	50 Preps
Fungal Protein Extration Buffer	50mL
Fungal Suspension Buffer	8mL
Lyticase (10U/μL)	5000U

Storage

Store Lyticase at -20°C and other kit components at 2-8°C. Stable for 12 months when stored and used as recommended.

Chemical required but not supplied

β- mercaptoethanol

Protocol

1. Pellet fungal cells (culture OD_{600} 1.5–2.0) by centrifugation at 3000 × g for 10 minutes. Suspend the cell pellet in an equal volume of the Fungal Suspension Buffer.
   Add 1μL of β-mercaptoethanol per 100μL fungal suspension.

2. Vortex for 1 minute or until the cell suspension is homogeneous. Incubate the suspension for 5 minutes at 4°C. Vortex it again to suspend the cells.
3. Spin down the Lyticase vial gently to bring the solution to the bottom. Add 10U Lyticase for each 120µL cell suspension. Gently mix the content.
4. Incubate the suspension at 37°C for 30–60 minutes. Lysis can be monitored by taking 25µL suspension, mixing with 1mL Fungal Protein Extraction Buffer and reading optical density at 800nm.
5. At the end of incubation, centrifuge the suspension at 10,000 × g for 5 minutes. Remove and discard the supernatant carefully, leaving the spheroplast pellet in the tube.
   Optional: Add 5–10 volume of the Fungal Suspension Buffer to the spheroplast pellet. Resuspend the spheroplast by gently tapping the tube. Centrifuge again as above and discard the supernatant.
6. Lysis: Suspend the fungal pellet (now spheroplast) in an appropriate volume of the Fungal Protein Extraction Buffer (2–3 times the volume of cell pellet). Pipette the suspension up and down a few times. Vortex periodically and incubate on ice for 30 minutes. Incubating the cells for 1–3 minutes at 37°C or a brief sonication step may further facilitate the lysis. Sonication is necessary for shearing genomic DNA.
   Optional: To prevent protease activity, add Protease Inhibitor Cocktail (100X) (Cat#P6730; Not supplied) in lysis buffer at a ratio of 1:100 and mix well.
7. Centrifuge at 20,000 × g for 30 minutes at 4°C. Collect clear lysate. The lysate is now ready for purification of protein, other applications, or further analysis.