Minute™ Yeast/Fungus Nuclear and Cytoplasmic Protein Extraction Kit

Catalog Number: YN-058 (20 Preps)

Introduction

The Minute™ Yeast/Fungus Nuclear and Cytoplasmic Protein Extraction Kit offers a fast and reliable method to isolate both cytosolic and nuclear proteins from yeast and fungus. This easy-to-use kit allows you to separate these crucial cellular components in about 1h, streamlining your research workflow. The kit provides all the necessary buffers and disposable tools for an efficient protein extraction. Using this kit, you'll have access to both cytosolic and intact nuclei that can be used for nuclear protein and nucleic acid extraction.

Kit components

1.	Buffer A	20 ml
2.	Buffer B	20 ml
3.	Buffer C	2 ml
4.	Protein extraction powder	5g
5.	pestles for 1.5 ml microfuge tube	2
6.	1.5 ml microfuge tube	20

Storage: Store buffer B at -20°C and rest of the kit at room temperature

Additional Materials Required

Table-Top Microcentrifuge, ddH2O

Important Product Information

Prior to nuclear isolation, thaw out buffer B at room temperature and place on ice. Addition of protease inhibitor cocktail to aliquot of buffer A is recommended. For determination of protein concentration, BCA kit (Pierce) is recommended. To study protein phosphorylation, **phosphatase inhibitors** (such as PhosStop from Roche) should be added to buffer A and B prior to use. Perform all centrifugations from step 4-8 at 4°C.

Protocol

1. Harvest yeast/fungus cells in log growth phase by centrifuging at 10,000 X g for 5 min. Collect cells in a 1.5 ml microfuge tube provided. Make sure that the wet volume of pellet is between 100-120 µl. The volume can be easily estimated by comparing it to a 1.5 ml microfuge tube with 100 µl water.

2. Resuspend the pellet in 1 ml cold ddH2O and centrifuge at 10,000 X g for 3 min. Decant the supernatant. Add 150-200 mg protein extraction powder to the tube followed by addition of 1 ml cold ddH2O to the tube. Cap it and centrifuge at 10,000 X g for 3 min. **Remove and discard supernatant completely**.

3. Grinding the pellet repeatedly with the pestle provided for about 3-4 min (300-400 times) with back-and-forth twisting force. Add 400 µl buffer A to the tube and continue to grind for about thirty times (note: The pestle is reusable, for cleaning simply soak it in bleach, rinse with water and dry it with paper towel).

4. Centrifuge the tube at 3,000 X g for 5 min at 4ºC. Transfer all supernatant to a fresh pre-chilled microfuge tube from your lab and place on ice. Repeat step 3 one more time and combine the supernatants (800 µl) in the microfuge tube on ice.

5. Centrifuge the combined supernatants at 3,000 X g for 3 min and carefully transfer supernatant to a fresh 1.5 ml microfuge tube (this is total cell lysate fraction) without disturbing the pellet. Discard the pellet

6. Centrifuge total cell lysate at 10,000 X g for 10 min. Transfer supernatant to a fresh 1.5 ml microfuge tube and centrifuged at 16,000 X g for 30 min. The cell wall material is floating on the top, which can be removed by pipetting. Transfer clear supernatant to a fresh tube. This is cytosolic fraction.

7. Resuspend the pellet from step 6 after 10,000 X g spin in 0.5 ml buffer B by pipetting up and down for 30-40 times add 50 µl buffer C (optional, see tech notes below) to tube and vortex briefly. Centrifuge at 10,000 X g for 10 min at 4ºC. Remove and discard supernatant.

8. Resuspend the pellet in 0.5 ml buffer B and 50 µl buffer C again as in step 7 by pipetting and centrifuge at 10,000 X g for 10 min. The pellet contains isolated nuclei that can be used for protein or nucleic acid extraction. Typically, the protein yield is about 50-80 ug/sample. Isolated nuclei fraction can be dissolved in detergent-containing buffers for downstream protein analysis. Reagents in following table are recommended some common applications.

Tech Notes

1. If excessive cytosolic protein contamination is observed, the centrifugal force in step 6 can be reduced from 10,000 X g to 8,000 X g for 10 min.
2. Both buffer A and B are detergent-free. Buffer B serves as a separation and low stringency washing buffer.
3. Buffer C contains non-ionic detergent that can increase the washing stringency of buffer B. If the presence of detergent is not desired, buffer C can be omitted.
4. If well-separated single nucleus is desired, the nuclear pellet in step 8 can be resuspend in 100-200 µl 1 X PBS containing 1% BSA and centrifuged at 100 X g for 3 min. Transfer supernatant to a fresh tube. This is single nuclei fraction.

Following protein solubilization reagents are recommended.

Product Name	Cat. No.	Applications
Minute™ Denaturing Protein Solubilization Reagent	WA-009	SDS-PAGE electrophoresis and Western blotting, trypsin digestion, purification of proteins with biotin labeling or histidine labeling, etc.
Minute™ Non-Denatured Protein Solubilization Reagent	WA-010	ELISA, immunoprecipitation/Co-IP, enzymatic activity determination and other applications.
Minute™ Protein Solubilization Reagent for MS	WA-011	Trypsin digestion and subsequent mass spectrometry analysis.