Research Square

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Rapid and reliable preparation of total cell lysates from fission yeast

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Method Article

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Abstract

Introduction

In the biochemical and molecular biological studies of the fission yeast _Schizosaccharomyces pombe_, the current methods that are mainly used for preparation of the total cell lysates are mostly laborious and sometimes even ineffective. We propose a method for successful and reproducible extraction of proteins from _S. pombe_ cells by slightly modifying the V. V. Kushnirov's protocol that previously demonstrated effective extraction of proteins from the two yeast species _Saccharomyces cerevisiae_ and _Hansenula polymorpha_.

Reagents

0.7 N NaOH solution SDS-PAGE sample buffer \(50 mM Tris-HCl \[pH 6.8], 2% SDS, 4% 100 mM DTT, 10% glycerol, 0.1% bromophenol blue) #Note: Glycerol and bromophenol blue are not necessarily required.

Equipment

Microcentrifuge Heating block \(or alternatives for heating samples at nearly 100˚C)

Procedure

1. Culture _S. pombe_ cells in an appropriate medium. 2. Harvest cells in a collection tube. 3. Suspend the cells with medium that was used in the culture of the cells \(do not exceed the half volume of the tube). 4. Add an equal amount of 0.7 N NaOH solution and mix well. 5. Incubate at room temperature for 3 minutes. 6. Centrifuge the tube \(5,000 rpm, 1 min) and discard the supernatant. 7. Add SDS-PAGE sample buffer and mix well. 8. Heat at 95-100˚C for 5 min. 9. Centrifuge at at least 3,500 rpm, and use the supernatant as the total cell lysate.

Timing

Within 20 minutes

Troubleshooting

Longer incubation at 95-100˚C will reduce a protein yield.

Anticipated Results

Almost all proteins are expected to be extracted. Since proteins are extracted with SDS-PAGE sample buffer, you can directly use the samples for SDS-PAGE or other experiments.

References

Kushnirov, V.V. Rapid and reliable protein extraction from yeast. _Yeast_ **16**, 857-860 \(2000).

Figures

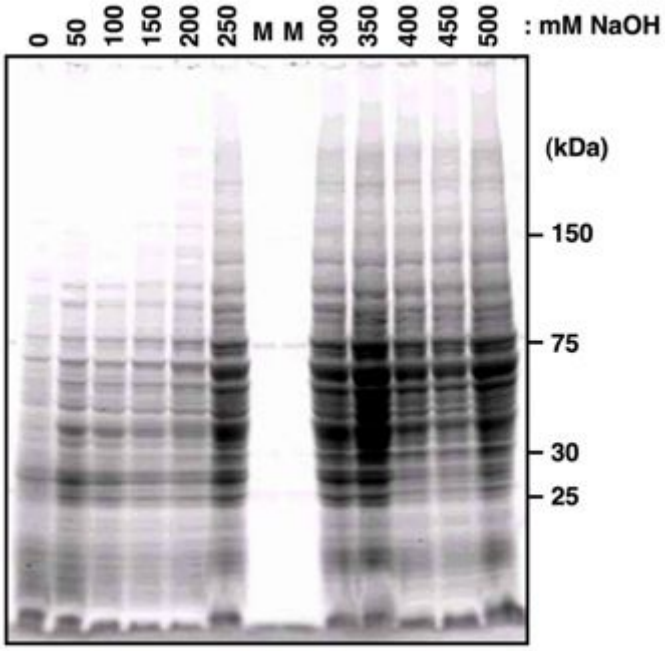

Figure 1

S. pombe cells were grown on minimum solid medium, harvested in MM liquid medium, and pretreated with indicated concentration of NaOH. Proteins were subsequently extracted with SDS-PAGE sample buffer, resolved by SDS-PAGE and stained with Coomassie blu