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Version 2

Yeast Crude Protein Extraction V.2

DOI

<https://dx.doi.org/10.17504/protocols.io.kqfcvtn>

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Yeast Protocols, Tools, a...



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Protocol status: Working



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Abstract

Quick crude protein extraction from budding or fission yeast.

Safety warnings

 2-mercaptoethanol (also known as β -mercaptoethanol) is toxic and must be handled under a fume hood.

Before start

Cool NaOH on ice before use.



- 1 Grow cells to mid-exponential phase in liquid medium.
- 2 Normalize cells to an OD₆₀₀ of 2-3 in 1 mL of H₂O.
- 3 Centrifuge 1 mL of H₂O containing your cells at 9000 RPM for two minutes.
 00:02:00
- 4 Resuspend pelleted cells in 100 uL 0.2 M NaOH and incubate on ice for 15 minutes.
 100 µL
 00:15:00
- 5 Centrifuge cells at 9000 RPM for two minutes.
 00:02:00
- 6 Resuspend the pellet in 100 uL of Yeast Protein Extraction Sample Buffer.
 100 µL

Protocol

NAME

Yeast Protein Extraction Sample Buffer

CREATED BY

Alan Cone

[Preview](#)

- 6.1 Add 2.4 mL 1 M Tris pH 6.8
 2 µL
- 6.2 Add 20 mL 50% Glycerol
 20 µL
 Glycerol **Catalog #G5516**
- 6.3 Add 8 mL 10% SDS
 8 µL
- 6.4 Add 4 mL of 1% BPB



 4 μL

6.5 Add 65.6 mL H_2O to make 100 mL of Buffer

 66 μL

7 Add 0.4 μL 2-mercaptoethanol to the mixture.

 0.4 μL

8 Boil cells in sample buffer for 5 minutes at 95 C.

 00:05:00

9 Vortex briefly then centrifuge at 10,000 RPM for 3 minutes.

 00:03:00

10 Can store at -80 C or load supernatant into a gel.