VERSION 2

NOV 08, 2017

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.kqfcvtn

Protocol Citation: Alan Cone 2017. Yeast Crude Protein Extraction. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.kqfcvtn>

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Protocol status: Working

Created: Nov 08, 2017

Last Modified: Mar 14, 2018

PROTOCOL integer ID:
 8679

Yeast Crude Protein Extraction V.2

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Yeast Protocols, Tools, and Tips

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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 INSTRUCTIONS

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 J Cone

PREVIEW

6.1 Add 2.4 mL 1 M Tris pH 6.8

2 mL

6.2 Add 20 mL 50% Glycerol

20 mL

Glycerol Contributed by users Catalog #G5516

6.3 Add 8 mL 10% SDS

8 mL

6.4 Add 4 mL of 1% BPB

4 mL

6.5 Add 65.6 mL H₂O to make 100 mL of Buffer

66 mL

7 Add 0.4 uL 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.