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Yeast Crude Protein Extraction V.2

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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 OD_{600} of 2-3 in 1 mL of H_2O.

3 Centrifuge 1 mL of H_2O 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

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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 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.