

Yeast Crude Protein Extraction Version 2

Alan Cone

Abstract

Quick crude protein extraction from budding or fission yeast.

Citation: Alan Cone Yeast Crude Protein Extraction. **protocols.io**

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

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Before start

Cool NaOH on ice before use.

Protocol

Step 1.

Grow cells to mid-exponential phase in liquid medium.

Step 2.

Normalize cells to an OD₆₀₀ of 2-3 in 1 mL of H₂O.

Step 3.

Centrifuge 1 mL of H₂O containing your cells at 9000 RPM for two minutes.

 DURATION

00:02:00

Step 4.

Resuspend pelleted cells in 100 uL 0.2 M NaOH and incubate on ice for 15 minutes.

 AMOUNT

100 µl Additional info:

 DURATION

00:15:00

Step 5.

Centrifuge cells at 9000 RPM for two minutes.

 DURATION

00:02:00

Step 6.

Resuspend the pellet in 100 uL of Yeast Protein Extraction Sample Buffer.

 AMOUNT

100 µl Additional info:

 PROTOCOL

Yeast Protein Extraction Sample Buffer

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Step 6.1.

Add 2.4 mL 1 M Tris pH 6.8

 [AMOUNT](#)

2 ml Additional info:

Step 6.2.

Add 20 mL 50% Glycerol

 [AMOUNT](#)

20 ml Additional info:



[REAGENTS](#)

✓ Glycerol [G5516](#) by Contributed by users

Step 6.3.

Add 8 mL 10% SDS

 [AMOUNT](#)

8 ml Additional info:

Step 6.4.

Add 4 mL of 1% BPB

 [AMOUNT](#)

4 ml Additional info:

Step 6.5.

Add 65.6 mL H₂O to make 100 mL of Buffer

 [AMOUNT](#)

66 ml Additional info:

Step 7.

Add 0.4 µL 2-mercaptoethanol to the mixture.

 [AMOUNT](#)

0.4 µl Additional info:

Step 8.

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

 [DURATION](#)

00:05:00

Step 9.

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

 [DURATION](#)

00:03:00

Step 10.

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

Warnings

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