Fungal DNA Lysis Buffer

Adam Taranto

Abstract

Lysis buffer for extraction of DNA from fungal material. Contains ionic and non-ionic detergents. Contains RNAse-A.

Composition:

NaCl	150mM
EDTA pH 8.0	1 mM
Tris Base pH 8.0	10 mM
Polyvinylpolypyrrolidone (PVPP)	2%
IGEPAL-CA630	0.5%
Sodium deoxycholate	0.5%
SDS	1%
RNAse-A	100 µg/mL

Citation: Adam Taranto Fungal DNA Lysis Buffer. **protocols.io**
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Materials

0.5mm diameter glass beads SI-BG05 by Scientific Industries, Inc.

IGEPAL-CA630 I3021 SIGMA-ALDRICH by Sigma Aldrich

- Tris(Hydroxymethyl)aminomethane PubChem CID: 6503 by Contributed by users
- Sodium Dodecyl Sulfate PubChem CID: 3423265 by Contributed by users
- EDTA Disodium Salt PubChem CID: 8759 by Contributed by users
- Sodium Chloride PubChem CID: 5234 by Contributed by users
- Sodium Deoxycholate PubChem CID: 23668196 by Contributed by users

RNase A (10 mg/mL) EN0531 by Thermo Fisher Scientific

- Polyvinylpolypyrrolidone CID 6917 by Contributed by users

Protocol

Prepare Stock Solutions

Step 1.
10% SDS solution

Dissolve 10 g SDS in 80 mL ddH_2O
Adjust volume to 100 mL

AMOUNT
10 g Additional info:

REAGENTS
Sodium Dodecyl Sulfate PubChem CID: 3423265 by Contributed by users

Prepare Stock Solutions

Step 2.

1M Tris Base, pH 8.0

Dissolve 12.114g Tris-Base in 80 mL ddH_2O

pH to 8.0 with 10 M HCl
Adjust volume to 100mL

AMOUNT
12 g Additional info:

REAGENTS
Tris Hydroxymethylaminomethane PubChem CID: 6503 by Contributed by users

Prepare Stock Solutions

Step 3.

0.5 M EDTA, pH 8.0

Add 18.61 g of disodium EDTA•$2H_2O$ to 80 mL of ddH_2O
Adjust the pH to 8.0 with NaOH (20 NaOH pellets)
Adjust volume to 100 mL

AMOUNT
19 g Additional info:

REAGENTS
EDTA Disodium Salt PubChem CID: 8759 by Contributed by users

Prepare Stock Solutions

Step 4.

3 M NaCl

Dissolve 17.532 g NaCl in 80 mL ddH_2O
Adjust volume to 100 mL

AMOUNT
18 g Additional info:

REAGENTS
Sodium Chloride PubChem CID: 5234 by Contributed by users

Prepare Lysis Solution - 100 mL

Step 5.

Add 500µL IGEPAL-CA630 to 82.3 mL ddH_2O

Stir until dissolved

AMOUNT
500 µl Additional info:

REAGENTS
IGEPAL-CA630 I3021 SIGMA-ALDRICH by Sigma Aldrich

NOTES
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Final concentration: 0.5%

Prepare Lysis Solution - 100 mL
Step 6.

Add 0.5g Sodium deoxycholate

Stir until dissolved

REAGENTS
Sodium Deoxycholate PubChem CID: 23668196 by Contributed by users

NOTES
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Final concentration: 0.5%

Prepare Lysis Solution - 100 mL
Step 7.

Add PVPP

Add 2g Polyvinylpolypyrrolidone, stir until dissolved.

AMOUNT
2 g Additional info:

REAGENTS
Polyvinylpolypyrrolidone CID 6917 by Contributed by users

NOTES
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Final concentration 2%

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PVPP precipitates phenols, tannins, alkaloids which may inhibit enzymes or damage DNA

Prepare Lysis Solution - 100 mL
Step 8.

Add 10 mL of 10% SDS stock solution

Stir until dissolved

AMOUNT
10 ml Additional info:

REAGENTS
Sodium Dodecyl Sulfate PubChem CID: 3423265 by Contributed by users

NOTES
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Final concentration: 1%

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1% SDS = 34.67 mM

Prepare Lysis Solution - 100 mL

Step 9.

Add 5 mL of 3M NaCl stock solution

AMOUNT
5 ml Additional info:

REAGENTS
Sodium Chloride PubChem CID: 5234 by Contributed by users

NOTES
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Final concentration: 150 mM

Prepare Lysis Solution - 100 mL

Step 10.

Add 200 µL of 0.5 M EDTA (pH 8.0) stock solution

AMOUNT
200 µl Additional info:

REAGENTS
EDTA Disodium Salt PubChem CID: 8759 by Contributed by users

NOTES
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Final concentration: 1 mM

Prepare Lysis Solution - 100 mL

Step 11.

Add 1 mL Tris-Base (pH 8.0)

Stir until dissolved

AMOUNT
1 ml Additional info:

REAGENTS
Tris Hydroxymethylaminomethane PubChem CID: 6503 by Contributed by users

NOTES
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Final concentration: 10 mM

Prepare Lysis Solution - 100 mL

Step 12.

Add 1mL of RNase A (10 mg/mL) stock solution

AMOUNT
1 ml Additional info:

REAGENTS
RNase A (10 mg/mL) EN0531 by Thermo Fisher Scientific

NOTES
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Final concentration: 100 µg/mL

Prepare Lysis Solution - 100 mL

Step 13.

Store lysis solution at 4˚C