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- Trockeneiszuschlag
- Gefahrgutzuschlag
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ZYMO RESEARCH

DNA Purification Made Simple

YeaStar Genomic DNA Kit™

For genomic DNA isolation from a broad spectrum of fungus species.

Highlights

- Genomic DNA can be used directly for Southern Blots, PCR, restriction enzyme digestion, etc.
- Fast spin column procedure yields pure yeast genomic DNA.
- No glass beads or phenol.
- Efficient DNA isolation from a broad spectrum of fungus species:

  Aspergills fumigatus

  Aspergills nidulans

  Aspergills nivens var. aureus

  Candida albicans

  Pichia pastoris

  Saccharomyces cerevisiae

  Schizosaccharomyces pombe

Catalog Numbers:
D2002

Scan with your smart-phone camera to view the online protocol/video.

Table of Contents

Product Contents

YeaStar Genomic DNA Kit™	D2002 (40 Preps.)	Storage
R-Zymolyase™ [1] (Lyophilized) Resuspend the lyophilized enzyme by adding 200 µl of the supplied Storage Buffer.	1,000 Units	Shipped at room temperature. Store at -20ºC after arrival.
YD Digestion Buffer	4.8 ml	Room Temp.
YD Lysis Buffer[2]	4.8 ml	Room Temp.
DNA Wash Buffer[3] (concentrate)	6 ml	Room Temp.
Zymo-Spin™ III Columns	40	Room Temp.
Collection Tubes	40	Room Temp.
Instruction Manual	1	-

1 This reagent contains beta-mercaptoethanol.
2 Contains Chaotropic salt. Irritant. Handle with care.
3 Add 24 ml of 95-100% Ethanol before use. Ethanol is not provided.

Product Description

The **YeaStar Genomic DNA Kit™** is designed for reliable and efficient isolation of genomic DNA from a broad spectrum of fungus species, including *Aspergills fumigatus*, *Aspergills nidulans*, *Aspergills nivens var. aureus*, *Candida albicans*, *Pichia pastoris*, *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, and any fungi whose cell walls are susceptible to yeast lytic enzyme lysis. The kit is based on highly efficient enzyme lysis and fast spin column technology. Each standard prep yields about 7-20 μg of DNA with a size distribution of 35-60 kb. The resulting genomic DNA can be used directly for all molecular biology analysis such as Southern Blot, PCR, restriction enzyme digestion, etc.

Protocol

Buffer Preparation

- ✓ *Before starting:* Add 24 ml of 95-100% ethanol to the **DNA Wash Buffer**. Protocol I and II are almost same, except that chloroform is used in Protocol I. Protocol I usually gives more recovery of DNA by 30-80% compared to Protocol II. Protocol II is chloroform-free. Chloroform is not provided.

- ✓ Add 200 µl of the supplied **Storage Buffer** to the lyophilized **R-Zymolyase™.** Mix to dissolve the enzyme completely, spin briefly in a micro-centrifuge. Store the reconstituted **R-Zymolyase™** at -20°C.

Protocol I

The kit works with either fresh cells or aged cells in either plates or liquid cultures. The following procedure is based on 1-1.5 ml culture (1-5 x 10^7 cells). Increasing the amount of cells above the recommended level may cause overloading of the system.

1. Spin 1-1.5 ml of cells down briefly or centrifuge at 500 g for 2 minutes. Remove the supernatant completely.

2. Add 120 µl of **YD Digestion Buffer** and 5 µl of **R-Zymolyase™** [1] (RNase A + Zymolyase™). Resuspend the pellet by vortexing and incubate at 37°C for 40-60 minutes.

3. Add 120 µl of **YD Lysis Buffer**[2]. Mix well by gently vortexing.

   Note: You can vortex hard for 10-20 seconds after adding **YD Lysis Buffer**. This will increase your DNA recovery but may result in shorter genomic DNA ranging from 20-35 kb. However, most of the DNA will remain more than 35 kb.

4. Add 250 µl of chloroform. Mix thoroughly for 1 minute.

5. Centrifuge in a table top centrifuge at >10,000 rpm for 2 minutes.

6. Load the supernatant onto the **Zymo-Spin™ III Column** and centrifuge at >10,000 rpm for 1 minute.

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7. Add 300 µl of **DNA Wash Buffer** and centrifuge for 1 minute at ≥10,000 rpm to wash. Add another 300 µl of **DNA Wash Buffer** to repeat the wash and centrifuge for 1 minute.

8. Transfer the **Zymo-Spin™ III Column** to a new 1.5 ml centrifuge tube and add 60 µl of water or TE directly onto the membrane. Wait for one minute then centrifuge for 10 seconds to elute the DNA.

Protocol II

Note: *Before starting*: Add 24 ml of 95-100% ethanol to the **DNA Wash Buffer.**

1. Spin 1-1.5 ml of cells down briefly or centrifuge at 500 g for 2 minutes. Remove the supernatant completely.

2. Add 120 µl of **YD Digestion Buffer** and 5 µl of **R-Zymolyase™**[1] (RNase A + Zymolyase™). Resuspend the pellet by vortexing and incubate at 37°C for 40-60 minutes.

3. Add 120 µl of **YD Lysis Buffer**[2]. Mix well by gently vortexing.

   Note: You can vortex hard for 10-20 seconds after adding **YD Lysis Buffer**. This will increase your DNA recovery but may result in shorter genomic DNA ranging from 20-35 kb. However, most of the DNA will remain more than 35 kb.

4. Centrifuge in a table top centrifuge at >10,000 rpm for 2 minutes.

5. Load the supernatant onto the **Zymo-Spin™ III Column** and centrifuge at >10,000 rpm for 1 minute.

6. Add 300 µl of **DNA Wash Buffer** and centrifuge for 1 minute at ≥10,000 rpm to wash. Add another 300 µl of **DNA Wash Buffer** to repeat the wash and centrifuge for 1 minute.

7. Transfer the **Zymo-Spin™ III Column** to a new 1.5 ml centrifuge tube and add 60 µl of water or TE directly onto the membrane. Wait for one minute then centrifuge for 10 seconds to elute the DNA.

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Troubleshooting

Several factors affect the yield of the **YeaStar Genomic DNA Kit™**. Here are some suggestions for obtaining optimal efficiency:

1. The initial amount of yeast cells used is important. The cultures of certain fungi strains can reach very high density. In this case using less volume of cells, such as using 0.4-0.8 ml instead of using 1-1.5 ml as we have suggested. Also, too many cells can easily overload the system. Try to use less cells when you suspect that cell lysis is incomplete. You should be able to see that cells are lysed after the incubation with the enzyme in step 2 of both Protocol I and II.

2. Fresh and early log phase cells are usually more susceptible to yeast lytic enzyme lysis than aged cells. Try to use fresh cultures whenever possible.

3. Susceptibility to yeast lytic enzymes varies for different yeast species. If you see incomplete lysis, extend the first incubation time up to 2 hours or over 16 hours.

Ordering Information

Product Description	Catalog No.	Size
YeaStar Genomic DNA Kit™	D2002	40 Preps.

Individual Kit Components	Catalog No.	Amount
R-Zymolyase™	E1006	1,000 Units (lyophilized) Supplied with 500 µl Storage Buffer
YD Digestion Buffer	D2002-1	4.8 ml
YD Lysis Buffer	D2002-2	4.8 ml
DNA Wash Buffer	D4003-2-6	6 ml
Zymo-Spin™ III Columns	C1005-50	50 Pack
Collection Tubes	C1001-50	50 Pack

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