Instructions

YeaStar Genomic DNA Kit™

Catalog No. **D2002**

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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

Help your fellow colleagues:

Have you sucessfully isolated DNA for different fungus strains using YeaStar Genomic DNA procedure?

Please email us with the strain information and we will update our strain list on instruction and web pages to help other researchers.

Thank you.

General Information

Package Contents:

		Storage conditions:
1	1,000 units R-Zymolyase™ ⊗ (Lyophilized) Resuspend the lyophilized enzyme by adding 200 ul of the supplied storage buffer	Shipped at room temperature. Store at -20ºC after arrival
1	4.8 ml YD Digestion Buffer	Room Temperature
1	4.8 ml YD Lysis Buffer*	Room Temperature
1	6 ml DNA Wash Buffer (Concentrated. Add 24 ml of 95-100% ethanol before use.†)	Room Temperature
1	40 Zymo-spin III columns	Room Temperature
1	40 2 ml Collection tubes	Room Temperature
1	Instruction sheet	Room Temperature

Reagents provided in this kit are designed for 40 fungus genomic DNA preparations.

⊗ This reagent contains beta-mercaptoethanol.
* Contains Chaotropic salt. Irritant. Handle with care.
† Ethanol is not provided.

Ordering Information:

Product	Cat. No.	Size
YeaStar Genomic DNA Kit	D2002	1 kit
For Individual Sale:		
R-Zymolyase™	E1006	1,000 Units (lyophilized) Supplied with 500 ul Storage Buffer

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.

Note: 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 ul 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

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™** ⊗ (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***. Mix well by gently vortexing.
   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.
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.

⊗ Contains beta-mercaptoethanol. * Contains Chaotropic salt. Irritant. Handle with care.

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

Protocol II

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™** ⊗ (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***. Mix well by gently vortexing.
   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.

⊗ Contains beta-mercaptoethanol. * Contains Chaotropic salt. Irritant. Handle with care.

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.