E.Z.N.A.® Yeast DNA Kit

D3370-00	5 preps
D3370-01	50 preps
D3370-02	200 preps

June 2013

E.Z.N.A.® Yeast DNA Kit

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OMEGA bio-tek

Innovations in nucleic acid isolation

Introduction

The E.Z.N.A.® Yeast DNA Kit allows for the rapid and reliable isolation of high-quality total cellular DNA from a wide variety of yeast species. Up to 3 mL log-phase culture (OD_{600} of 1.0 in YPD medium) can be processed. The system combines the reversible nucleic acid binding properties of HiBind® matrix with the speed and versatility of spin column technology to yield approximately 15-30 µg DNA with an A_{260}/A_{280} ratio of 1.7-1.9. Purified DNA is suitable for PCR, restriction enzyme digestion, and hybridization applications. There are no organic extractions thus reducing plastic waste and hands-on time to allow multiple samples to be processed in parallel.

Note: E.Z.N.A.® Yeast DNA Kit will isolate all cellular DNA, including plasmid DNA.

Overview

If using the E.Z.N.A.® Yeast DNA Kit for the first time, please read this manual before beginning the procedure. Yeast cells are grown to log-phase and spheroblasts are subsequently prepared. Following lysis, binding conditions are adjusted and the sample is applied to a HiBind® DNA Mini Column. Three rapid wash steps remove trace salts and protein contaminants. DNA is eluted in water or low ionic strength buffer. Purified DNA can be directly used in downstream applications without the need for further purification.

New in this Edition:

- HB Buffer has been replaced by HBC Buffer. Isopropanol is required and supplied by the user.
- Equilibration Buffer is no longer included with this kit. An optional Column Equilibration Protocol has been added to the protocol for your convenience.
- Equilibration Buffer is replaced with 3M NaOH provided by the user.

Kit Contents

Product	D3370-00	D3370-01	D3370-02
Purifications	5	50	200
HiBind® DNA Mini Columns	5	50	200
2 mL Collection Tubes	10	100	400
YL Buffer	1.5 mL	15 mL	60 mL
YDL Buffer*	1.5 mL	15 mL	60 mL
HBC Buffer	3 mL	25 mL	100 mL
DNA Wash Buffer	2 mL	15 mL	3 x 20 mL
Glass Beads	300 mg	3 g	12 g
Elution Buffer	1.5 mL	15 mL	60 mL
SE Buffer	3 mL	30 mL	120 mL
Lyticase (units)	250	2,500	4 x 2,500
Proteinase K Solution	150 µL	1.1 mL	4 x 1.1 mL
RNase A	30 µL	275 µL	1.1 mL
User Manual	✓	✓	✓

*YDL Buffer contains a chaotropic salt.

Storage and Stability

All components of the E.Z.N.A.® Yeast DNA Kit, except the RNase A and lyticase can be stored at 22-25°C and are guaranteed for at least 24 months from the date of purchase. Proteinase K Solution can be stored at room temperature for 12 months. For long-term storage (>12 months), store Proteinase K Solution at 2-8°C. Store RNase A at -20°C. Under cool ambient conditions, a precipitate may form in the YL Buffer and/or YDL Buffer. If a precipitate is present, heat the bottle at 37°C to dissolve.

Preparing Reagents

1. Prepare SE Buffer with β-mercaptoethanol. Add 10 µL β-mercaptoethanol per 1 mL SE Buffer. The mixture is stable for 1 month at room temperature.

2. Prepare a lyticase stock solution at 2,500 units/mL and aliquot. Store aliquots at -20°C.

Kit	SE Buffer to be Added
D3370-00	100 µL
D3370-01	1 mL
D3370-02	1 mL per tube

3. Dilute DNA Wash Buffer with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
D3370-00	8 mL
D3370-01	60 mL
D3370-02	80 mL per bottle

4. Dilute HBC Buffer with isopropanol as follows and store at room temperature.

Kit	Isopropanol to be Added
D3370-00	1.2 mL
D3370-01	10 mL
D3370-02	40 mL

Recommended Settings

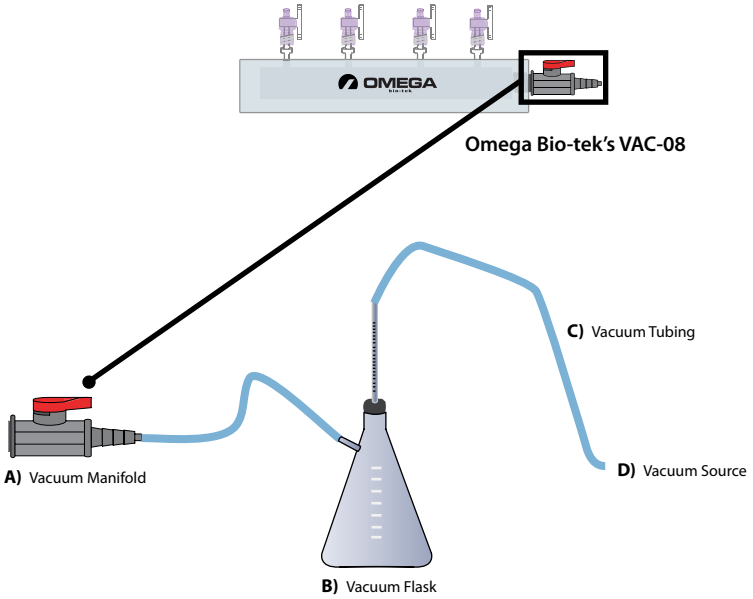
The following is required for use with the Vacuum Protocol:

A) Vacuum Manifold (We recommend Omega Bio-tek's VAC-08)
Other Compatible Vacuum Manifolds: Qiagen QIAvac24, Sigma Aldrich VM20, Promega Vacman®, or manifold with standard Luer connector
B) Vacuum Flask
C) Vacuum Tubing
D) Vacuum Source (review tables below for pressure settings)

Manifold	Recommended Pressure (mbar)
VAC-08	-200 to -600

Conversion from millibars:	Multiply by:
millimeters of mercury (mmHg)	0.75
kilopascals (kPa)	0.1
inches of mercury (inHg)	0.0295
Torrs (Torr)	0.75
atmospheres (atm)	0.000987
pounds per square inch (psi)	0.0145

Illustrated Vacuum Setup:



E.Z.N.A.® Yeast DNA Kit Protocol - Centrifugation Protocol

This method isolates genomic DNA from up to 3 mL yeast culture (<2 x 10^7 cells). All centrifugation steps should be performed at room temperature.

Materials and Equipment to be Supplied by User:

- Tabletop microcentrifuge capable of at least 10,000 x *g*
- Nuclease-free 1.5 mL microcentrifuge tubes
- Incubators or water baths capable of at least 65°C
- Shaking water bath capable of at least 55°C
- 100% Ethanol
- Isopropanol
- β-mercatopethanol
- Optional: 3M NaOH

Before Starting:

- Prepare the SE Buffer, lyticase solution, and DNA Wash Buffer according to the instructions in the "Preparing Reagents" section on Page 4.
- Set the water baths and/or incubators to 30°C and 65°C.
- Set the shaking water bath to 55°C.
- Heat the Elution Buffer to 65°C.

1. Culture yeast in YPD medium to an OD_{600} of 1.0.

2. Centrifuge ≤3 mL culture (< 2 x 10^7) at 4,000 x *g* for 10 minutes.

3. Aspirate and discard the medium.

4. Resuspend cells in 480 µL SE Buffer and 20 µL lyticase solution.

   Note: β-mercaptoethanol must be added to the SE Buffer before preparing the lyticase solution. Please refer to the "Preparing Reagents" section on Page 4 for instructions.

5. Incubate at 30°C for at least 30 minutes.

6. Centrifuge at 500 x *g* for 10 minutes to pellet the spheroblasts.

7. Resuspend cells in 200 µL YL Buffer.

8. Add 50 mg glass beads. Vortex for 5 minutes.

9. Add 20 µL Proteinase K Solution. Vortex to mix well.

10. Incubate at 55°C in a shaking water bath to completely lysis the cells.

    Note: Usually no more than 1 hour is required for lysis. If a shaking water bath is not available, incubate and shake or briefly vortex the samples every 20-30 minutes.

11. Add 5 µL RNase A. Invert several times to mix.

12. Let sit at room temperature for 5 minutes.

Optional: Centrifuge at 10,000 x *g* for 5 minutes to pellet insoluble debris. Carefully aspirate the supernatant and transfer to a clean microcentrifuge tube leaving behind any insoluble pellet.

13. Add 220 µL YDL Buffer. Vortex at maximum speed for 15 seconds.

    Note: A wispy precipitate may form upon addition of YDL Buffer; it does not interfere with DNA recovery.

14. Incubate at 65°C for 10 minutes.

15. Add 220 µL 100% ethanol. Vortex at maximum speed for 20 seconds. If any precipitates can be seen at this point, break the precipitates by pipetting up and down 10 times.

16. Insert a HiBind® DNA Mini Column into a 2 mL Collection Tube.

Optional Protocol for Column Equilibration:

1. Add 100 µL 3M NaOH to the HiBind® DNA Mini Column.
2. Centrifuge at maximum speed for 30-60 seconds.
3. Discard the filtrate and reuse the collection tube.

17. Transfer the entire sample from Step 15 to the column, including any precipitates that may have formed.

18. Centrifuge at 10,000 x *g* for 1 minute.

19. Discard the filtrate and the Collection Tube.

20. Insert the HiBind® DNA Mini Column into a new 2 mL Collection Tube.

21. Add 500 µL HBC Buffer.

    Note: HBC Buffer must be diluted with isopropanol before use. Please see Page 4 for instructions.

22. Centrifuge at 10,000 x *g* for 1 minute.

23. Discard the filtrate and reuse the Collection Tube.

24. Add 700 µL DNA Wash Buffer..

    Note: DNA Wash Buffer must be diluted with 100% ethanol according to the instructions in the "Preparing Reagents" section on Page 4.

25. Centrifuge at 10,000 x *g* for 1 minute.

26. Discard the filtrate and reuse the Collection Tube.

27. Repeat Steps 24-26 for a second DNA Wash Buffer wash step.

28. Centrifuge the empty HiBind® DNA Mini Column for 2 minutes at maximum speed (≥10,000 x *g*) to dry the column matrix.

    Note: It is important to dry the column membrane before elution. Residual ethanol may interfere with downstream applications.

29. Transfer the HiBind® DNA Mini Column into a nuclease-free 1.5 mL microcentrifuge tube.

30. Add 50-100 µL Elution Buffer heated to 65°C.

31. Let sit at room temperature for 3 to 5 minutes.

    Note: Incubating the HiBind® DNA Mini Column at 65°C rather than room temperature will give a modest increase in DNA yield per elution.

32. Centrifuge at 10,000 x *g* for 1 minute.

33. Repeat Steps 30-32 for a second elution step.

    Note: Any combination of the following steps can be used to help increase DNA yield.

    - After adding the Elution Buffer, incubate the column for 5 minutes.
    - Increase the elution volume.
    - Repeat the elution step with fresh Elution Buffer (this may increase the yield, but decrease the concentration).
    - Repeat the elution step using the eluate from the first elution (this may increase yield while maintaining elution volume).

34. Store DNA at -20°C.

E.Z.N.A.® Yeast DNA Kit Protocol - Vacuum Protocol

Please read through previous section of this manual before using this protocol.

Materials and Equipment to be Supplied by User:

- Vacuum manifold for microcentrifuge tubes
- Vacuum source
- Tabletop microcentrifuge capable of at least 10,000 x *g*
- Nuclease-free 1.5 mL microcentrifuge tubes
- Incubators or water baths capable of at least 65°C
- Shaking water bath capable of at least 55°C
- 100% Ethanol
- Isopropanol
- β-mercatopethanol
- Optional: 3M NaOH

Before Starting:

- Prepare the SE Buffer, lyticase solution, and DNA Wash Buffer according to the instructions in the "Preparing Reagents" section on Page 4.
- Set the water baths and/or incubators to 30°C and 65°C.
- Set the shaking water bath to 55°C.
- Heat the Elution Buffer to 65°C.

1. Culture yeast in YPD medium to an OD_{600} of 1.0.

2. Centrifuge ≤3 mL culture (< 2 x 10^7) at 4,000 x *g* for 10 minutes.

3. Aspirate and discard the medium.

4. Resuspend cells in 480 µL SE Buffer and 20 µL lyticase solution.

   Note: β-mercaptoethanol must be added to the SE Buffer before preparing the lyticase solution. Please refer to the "Preparing Reagents" section on Page 4 for instructions.

5. Incubate at 30°C for at least 30 minutes.

6. Centrifuge at 500 x *g* for 10 minutes to pellet the spheroblasts.

7. Resuspend cells in 200 µL YL Buffer.

8. Add 50 mg glass beads. Vortex for 5 minutes.

9. Add 20 µL Proteinase K Solution. Vortex to mix well.

10. Incubate at 55°C in a shaking water bath to completely lysis the cells.

    Note: Usually no more than 1 hour is required for lysis. If a shaking water bath is not available, incubate and shake or briefly vortex the samples every 20-30 minutes.

11. Add 5 µL RNase A. Invert several times to mix.

12. Let sit at room temperature for 5 minutes.

Optional: Centrifuge at 10,000 x *g* for 5 minutes to pellet insoluble debris. Carefully aspirate the supernatant and transfer to a clean microcentrifuge tube leaving behind any insoluble pellet.

13. Add 220 µL YDL Buffer. Vortex at maximum speed for 15 seconds.

    Note: A wispy precipitate may form upon addition of YDL Buffer; it does not interfere with DNA recovery.

14. Incubate at 65°C for 10 minutes.

15. Add 220 µL 100% ethanol. Vortex at maximum speed for 20 seconds. If any precipitates can be seen at this point, break the precipitates by pipetting up and down 10 times.

16. Prepare the vacuum manifold according to manufacturer's instructions and connect the HiBind® DNA Mini Column to the manifold.

Optional Protocol for Column Equilibration:

1. Add 100 µL 3M NaOH to the HiBind® DNA Mini Column.
2. Turn on the vacuum source to draw the NaOH through the column.
3. Turn off the vacuum.

17. Transfer the entire sample from Step 15 to the HiBind® DNA Mini Column, including any precipitate that may have formed.

18. Turn on vacuum source to draw the sample through the column.

19. Turn off the vacuum.

20. Add 500 µL HBC Buffer to the HiBind® DNA Mini Column.

    Note: HBC Buffer must be diluted with isopropanol before use. Please see Page 4 for instructions.

21. Turn on vacuum source to draw the HBC Buffer through the column.

22. Turn off the vacuum.

23. Add 700 µL DNA Wash Buffer to the HiBind® DNA Mini Column.

    Note: DNA Wash Buffer must be diluted with ethanol before use. Please see Page 4 for instructions.

24. Turn on vacuum source to draw the DNA Wash Buffer through the column.

25. Turn off the vacuum.

26. Repeat Steps 23-25 for a second DNA Wash step.

27. Remove the HiBind® DNA Mini Column from the vacuum manifold and transfer to a new 2 mL Collection Tube.

28. Centrifuge the empty HiBind® DNA Mini Column for 2 minutes at maximum speed (≥10,000 x *g*) to dry the column matrix.

    Note: It is important to dry the column membrane before elution. Residual ethanol may interfere with downstream applications.

29. Transfer the HiBind® DNA Mini Column into a nuclease-free 1.5 mL microcentrifuge tube.

30. Add 50-100 µL Elution Buffer heated to 65°C.

31. Let sit at room temperature for 3 to 5 minutes.

    Note: Incubating the HiBind® DNA Mini Column at 65°C rather than room temperature will give a modest increase in DNA yield per elution.

32. Centrifuge at 10,000 x *g* for 1 minute.

33. Repeat Steps 30-32 for a second elution step.

    Note: Any combination of the following steps can be used to help increase DNA yield.

    - After adding the Elution Buffer, incubate the column for 5 minutes.
    - Increase the elution volume.
    - Repeat the elution step with fresh Elution Buffer (this may increase the yield, but decrease the concentration).
    - Repeat the elution step using the eluate from the first elution (this may increase yield while maintaining elution volume).

34. Store DNA at -20°C.

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact the technical support staff, toll free, at **1-800-832-8896**.

Problem	Cause	Solution
Clogged column	Incomplete lysis	Add the correct volume of YL Buffer and incubate at 55°C to obtain complete lysis. It may be necessary to extend incubation time to 30 minutes.
	Sample too large	Do not use greater than 3 mL culture at OD_{600} of 1.0 or 2 x 10^7 cell per spin column. For larger volumes, divide sample into multiple tubes.
	Incomplete removal of cell wall	Add more lyticase or extend the incubation time. It may be necessary to increase the incubation by 60 minutes.
Low DNA yield	Clogged column	See above
	Poor elution	Repeat elution or increase elution volume. Incubation of column at 65°C for 5 minutes after addition of Elution Buffer may increase yields.
	Improper washing	DNA Wash Buffer must be diluted with 100% ethanol.
Low A_{260}/A_{280} ratio	Extended centrifugation during elution step	Resin from the column may be present in eluate. Avoid centrifugation at speeds higher than specified. The material can be removed from the eluate by centrifugation; it will not interfere with PCR or restriction digests.
	Incomplete mixing with YDL Buffer	Repeat the procedure, this time making sure to immediately vortex the sample with YDL Buffer.
	Insufficient incubation	Increase incubation time with YL Buffer. Ensure that no visible cell clumps remain.

Problem	Cause	Solution
No DNA eluted	Poor cell lysis due to improper mixing with YDL Buffer	Mix thoroughly with YDL Buffer and incubate at 70°C prior to adding ethanol.
	Incomplete spheroblasting	Add more lyticase or extend the incubation time. It may be necessary to increase the incubation by 60 minutes.
	Ethanol not added to lysate/YDL Buffer mixture	Before applying sample to column, an aliquot of ethanol must be added. See protocol above.
	Ethanol was not added to DNA Wash Buffer	Dilute Wash Buffer with the indicated volume of ethanol before use.

Ordering Information

The following components are available for purchase separately.
(Call Toll Free at 1-800-832-8896)

Product	Part Number
Vacuum Manifold	VAC-08
YL Buffer, 100 mL	PD091
DNA Wash Buffer, 100 mL	PS010
Elution Buffer, 100 mL	PDR048
RNase A, 25 mg/mL, 400 µL	AC117