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Introduction

The E.Z.N.A.® Yeast DNA Kit allows rapid and reliable isolation of high-quality total cellular DNA from a wide variety of yeast species. Up to 3 ml of 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 of DNA with an A_{260}/A_{280} ratio of 1.7-1.9. Purified DNA is suitable for PCR, restriction digestion, and hybridization techniques. 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 speroblasts are subsequently prepared. Following lysis, binding conditions are adjusted and the sample applied to a HiBind® DNA Mini column. Two rapid wash steps remove trace salt and protein contaminants and finally 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.

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°C-25°C and are guaranteed for at least 24 months from the dated of purchase. Proteinase K should be stored at 15°C - 25°C. Lyticase must be stored at -20°C. Store RNase A at -20°C. Under cool ambient conditions, a precipitate may form in the Buffer YDL/YL. In case of such an event, heat the bottle at 37°C to dissolve. Store Buffer YDL/YL at room temperature.

Binding Capacity

Each HiBind® DNA Mini column can bind approximately 100 µg genomic DNA. Using greater than 2 x 10^7 Yeast cells is not recommended.

Kit Contents

Product	D3370-00	D3370-01	D3370-02
Purification times	5 Preps	50 Preps	200 Preps
HiBind® DNA Mini Columns	5	50	200
2 ml Collection Tubes	10	100	400
Buffer YL	1.5 ml	20 ml	50 ml
Buffer YDL	2 ml	20 ml	50 ml
Buffer HB	3 ml	30 ml	110 ml
DNA Wash Buffer	2 ml	20 ml	3 x 20 ml
Glass Beads (0.4-0.6mm)	300 mg	3 g	12 g
Elution Buffer	1 ml	10 ml	40 ml
Buffer SE	3 ml	30 ml	110 ml
Lyticase	250 units	2500 units	4x2500 units
Proteinase K	150 µl	1.4 ml	4 x 1.4 ml
RNase A	30 µl	275 µl	1.1 ml
User Manual	1	1	1

Buffer YDL contains a chaotropic salt. Use gloves and protective eyeware when handling this solution.

Materials to Be Provided by User

- Tabletop microcentrifuge and nuclease-free 1.5 ml tubes
- Water bath set to 30°C
- Shaking water bath set to 55°C
- Absolute ethanol (96%-100%) - Do not use other alcohols
- 2-mercaptoethanol

Before Starting

- Please read the entire booklet to become familiar with the E.Z.N.A.® Bacterial DNA Kit procedure.
- **Prepare a lyticase stock solution at 2500 Unit/ml and aliquot into adequate portions. Store each aliquot at -20°C and thaw before use**. Each sample will require 20 µl of this solution.

D3370-00	dissolve with 100 µl of Buffer SE
D3370-01	dissolve with 1 ml of Buffer SE
D3370-02	dissolve with 1 ml of Buffer SE for each tube

- Equilibrate Elution Buffer provided to 65°C.
- Dilute DNA Wash Buffer Concentrate with ethanol as follows and store at room temperature:

D3370-00	Add 8 ml absolute (96%-100%) ethanol
D3370-01	Add 80 ml absolute (96%-100%) ethanol
D3370-02	Add 80 ml absolute (96%-100%) ethanol/bottle
Store the diluted DNA Wash Buffer at room temperature.	

- ***Carry out all of centrifugation step at room temperature.***

E.Z.N.A.™ Yeast DNA Kit Spin Protocol

This method allows genomic DNA isolation from up to 3 ml yeast culture (< 2 x 10^7 cells).

1. Grow yeast culture in YPD medium to an OD_{600} of 1.0 . **Harvest no more than 3 ml culture (< 2 x 10^7) by centrifugation at 4,000 x g for 10 min at room temperature.**

2. **Discard medium and resuspend cells in 480 µl Buffer SE, 10 µl 2-mercaptoethanol and 20 µl lyticase solution.** Incubate at 30°C for at least 30 min.

3. Pellet spheroblasts by centrifuging 5 min at 4,000 x g at room temperature.

4. **Add 200 µl Buffer YL and 50 mg glass beads(0.4-0.6mm) to the sample. Vortex at maxi speed for 3-5 minutes.** Let it stand to allow the beads to settle. Transfer supernatant to a new 1.5 ml centrifuge tube.

5. **Add 25 µl Proteinase K solution and vortex to mix well**. Incubate at 65°C in a shaking water bath for 30 minutes.

6. Add 5 µl RNase A to the samples and invert tube several times to mix. Incubate at room temperature for 10 minutes.

7. (Optional) **Centrifuge at 10,000 x g for 5 min to pellet insoluble debris**. Carefully aspirate the supernatant and transfer to a sterile micro-centrifuge tube leaving behind any insoluble pellet.

8. **Add 220 µl Buffer YDL and 220 µl absolute ethanol to the sample and mix thoroughly by vortexing at maxi speed for 20 seconds.**

9. Assemble a HiBind® DNA Mini Column in a 2 ml collection (provided). **Transfer the entire sample from Step 8 into the column**, including any precipitate that may have formed. Centrifuge at 10,000 x g for 1 min to bind DNA. Discard the collection tube and filtrate.

10. **Place the column into a second 2 ml tube and wash by adding 500 µl Buffer HB.** Centrifuge at 10,000 x g for 1 min. Discard flow-through and reuse the collection tube.

11. **Place the column into the same collection tube and wash by adding 700 µl DNA Wash Buffer diluted with ethanol**. Centrifuge at 10,000 x g for 1 min. Discard flow-through and reuse the collection tube.

    NOTE: DNA Wash Buffer is supplied as a concentrate and must be diluted with absolute ethanol according to the instructions on Page 4, under "Before Starting."

12. **Wash the column with a second 700 µl DNA Wash Buffer and centrifuge as above**. Discard flow-through and reuse the collection tube.

13. **Using the same 2 ml collection tube, centrifuge HiBind® DNA Mini Column at maxi speed (≥10,000 x g) for 2 min to dry the column**.

    This step is critical for removal of trace ethanol that might otherwise interfere with down stream applications.

14. **Place the column into a nuclease-free 1.5 ml microfuge tube and add 50-100 µl of preheated (65°C) Elution Buffer to HiBind® DNA Mini Column matrix**. Allow columns to incubate for 3 to 5 min at room temperature after addition of Elution Buffer. NOTE: Incubating the HiBind® DNA column at 65°C rather than at room temperature prior to centrifugation will give a modest increase in DNA yield per elution.

15. **To elute DNA from the column, centrifuge at 10,000 x g for 1 min.**

16. Repeat the elution with a second 50-100 Elution Buffer.

    Note: Each 50-100 µl elution typically yields 60-70% of the DNA bound to the column. Thus two elutions generally give ~90%. However, increasing elution volume reduces the concentration of the final product. To obtain DNA at higher concentrations, elution can be carried out using 50 µl Elution Buffer (which slightly reduces overall DNA yield). Volumes lower than 50 µl greatly reduce yields. In some instances yields may be increased by incubating the column at 70°C (rather than at room temperature) upon addition of Elution Buffer.The expected yield from a 3 ml culture sample is 15-30 µg DNA depending on bacterial strain, medium, and growth phase.

E.Z.N.A.™ Yeast DNA Vacuum/Spin Protocol

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

1. Prepare samples by following the standard Protocol in previous section (Steps 1-10).

2. Prepare the vacuum manifold according to manufacturer's instructions and connect the V-Spin column to the manifold.

3. Load the sample/YDL/Ethanol mixture to the column. Switch on vacuum source to draw the sample through the column and turn off the vacuum.

4. Wash the column by adding 500 µl Buffer HB, draw the buffer HB through the column by turning on the vacuum source.

5. Wash the column by adding 700 µl DNA Wash Buffer, draw the wash buffer through the column by turning on the vacuum source. Repeat this step with another 650 µl DNA wash buffer.

6. Proceed step 15-18 of E.Z.N.A.™ Yeast DNA Spin Protocol on page 6-7.

Determination of Yield and Quality

The total DNA yield can be determined by a spectrophotometer using deionized water or Tris-HCl buffer as blank. DNA concentration is calculated as:

[DNA] = (Absorbance$_{260}$) x (0.05 µg/µl) x (Dilution factor)

The quality of DNA can be assessed by measuring absorbance at both 260 nm and at 280 nm. A ratio of (A_{260}/A_{280}) of 1.7-1.9 corresponds to 85%-95% purity. The expected yield from a 3 ml culture sample is 15-30 µg DNA depending on yeast strain, medium, and growth phase. If DNA is eluted with dH_2O rather than Tris buffer, store the sample at -20°C to prevent degradation.

Troubleshooting Guide

Problem	Possible Cause	Suggestions
Clogged column	Incomplete lysis	Add the correct volume of Buffer YL and incubate at 55°C to obtain complete lysis. It may be necessary to extend incubation time to 30 min.
	Sample too large	Do not use greater than 3 ml culture at OD_{600} 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 incubation by 60 min.
Low DNA yield	Clogged column	See above
	Poor elution	Repeat elution or increase elution volume (see note on page 6). Incubation of column at 65°C for 5 min after addition of Elution Buffer may increase yields.
	Improper washing	DNA Wash Buffer Concentrate must be diluted with absolute (96%-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 Buffer YDL	Repeat the procedure, this time making sure to vortex the sample with Buffer YDL immediately and completely.
	insufficient incubation.	Increase incubation time with BufferYL. Ensure that no visible cell clumps remain.
No DNA eluted	Poor cell lysis due to improper mixing with Buffer YDL.	Mix thoroughly with Buffer YDL 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 incubation by 60 min.
	Absolute ethanol not added to lysate/Buffer YDL mixture.	Before applying sample to column, an aliquot of ethanol must be added. See protocol above.
	No ethanol added to Wash Buffer.	Dilute Wash Buffer with the indicated volume of absolute ethanol before use.