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Introduction

E.Z.N.A.® Yeast DNA Kits allow 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 10 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.

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 spin-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.

New in this edition

- Newly introduced S-Spin column (D3370) & V-Spin column (D3570) features an attached cap which assures the elimination of potential contamination.
- Optional vacuum/spin protocol is available for V-Spin column

Storage and Stability

All components of the E.Z.N.A.® Yeast DNA Kit are stable for at least 24 months from date of purchase when stored at 22°C-25°C. During shipment, or storage in cool ambient conditions, precipitates may form in Buffer YDL. It is possible to dissolve such deposits by warming the solution at 37°C, though they do not interfere with overall performance.

Kit Contents

Product	D3470-00 D3370-00 D3570-00 Trial Sample	D3470-01 D3370-01 D3570-01 50 Preps	D3470-02 D3370-02 D3570-02 200 Preps
Components			
HiBind® DNA columns	5	50	200
2 ml Collection Tubes	15	150	600
Buffer YL	5 ml	20 ml	50 ml
Buffer YDL	5 ml	25 ml	100 ml
Buffer SE	3 ml	30 ml	110 ml
Proteinase K	140 µl	20 mg	80 mg
RNase A	30 µl	270 µl	1.1 ml
Lyticase	100 units	1000 units	4000 units
Wash Buffer Concentrate	12 ml	48 ml	2 x 60 ml
User Manual	1	1	1

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

Before Starting

- Please read the entire booklet to become familiar with the E.Z.N.A.® Yeast DNA Miniprep Kit procedure.
- Prepare a stock solution of Proteinase K (provided) at 15 mg/ml and aliquot into adequate portions. Store aliquots at -20°C.
- Prepare an RNase A stock solution (not provided) at 20 mg/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.
- Dilute DNA Wash Buffer Concentrate with ethanol as follows and **store at room temperature**.

D3370-00
D3470-00
D3570-00
Add 18 ml absolute (96%-100%) ethanol

D3370-01
D3470-01
D3570-01
Add 72 ml absolute (96%-100%) ethanol

D3370-02
D3470-02
D3570-01
Add 90 ml absolute (96%-100%) ethanol/bottle

Yeast DNA Miniprep Protocol

Have the following reagents and supplies ready:

- Tabletop microcentrifuge and nuclease-free 1.5 ml tubes.
- waterbath set to 30°C.
- Shaking waterbath set to 55°C.
- Sterile dH_2O (~0.5 ml per sample) equilibrated to 70°C.
- Absolute ethanol - do not use other alcohols.
- Rnase A stock solution (25 mg/ml); 5 µl per prep (optional)
- Reagents for Buffer SE below

This method allows genomic DNA isolation from up to 3 ml yeast culture.

1. Grow yeast culture in YPD medium to an OD_{600} of 10.

2. Harvest no more than 3 ml culture by centrifugation at 4,000 x g for 10 min at room temperature.

3. Discard medium and resuspend cells in 500 µl Buffer SE and add **20 units of Lyticase.** Incubate at 30°C for at least 30 min.
   NOTE: One vial lyticase (included) can be added to Buffer SE as follows, according to kit size: 2.8 ml Buffer SE (Trial Sample), 28 ml Buffer SE (50 preps), and 112 ml Buffer SE (200 preps).

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

5. Resuspend spheroblasts in **200 µl Buffer YL. Add 25 µl of a proteinase K solution at 15 mg/ml**. Vortex to mix well, and incubate at 55°C in a shaking waterbath to effect complete lysis. Usually no more than 1 h is required for spheroblast lysis. If no shaking waterbath is available, vortex the sample every 10 minutes.

6. **Add 5µl RNase A to the cell lysate. Centrifuge at 10,000 x g for 5 minutes. Transfer the supernatant to a new tube.**

7. Add 220 µl Buffer YDL and vortex to mix. Incubate at 70°C for 10 minutes. A wispy precipitate may form on addition of Buffer YDL, but does not interfere with DNA recovery.
8. Add 220 µl absolute ethanol and mix thoroughly by vortexing.

9. Assemble a HiBind® DNA spin-column in a 2 ml collection tube (provided). Transfer the entire sample from Step 7 into the column including any precipitate that may have formed. Centrifuge at 8,000 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 pipetting 650 µl of DNA Wash Buffer diluted with ethanol. Centrifuge at 8,000 x g for 1 min. Again, dispose of collection tube and flow-through.

    NOTE: DNA Wash Buffer is supplied as a concentrate and must be diluted with absolute ethanol according to the instructions on Page 3 under "**Before Starting**."

11. Using a **new collection tube**, wash the column with a second 650 µl of Wash Buffer and centrifuge as above. Discard flow-through.

12. Using the same empty 2ml collection tube, centrifuge the spin-column at maximum speed ($\geq$10,000 x g) for 2 min to dry the column. **This step is *critical* for removing trace amounts of ethanol; ethanol can lower yields and inhibit PCR.**

13. Place the column into a nuclease-free 1.5 ml microfuge tube and add 100-200 µl (depending on desired concentration) of preheated (70°C) sterile dH_2O or 10 mM Tris buffer, pH 8.5. Allow tubes to sit for 1 min at room temperature.

14. To elute DNA from the column, centrifuge at 8,000 x g for 1 min. Repeat the elution with a second 100-200 µl of pre-heated dH_2O if necessary.

NOTE: Incubation of the HiBind® DNA column loaded with 100-200 µl dH_2O or Tris buffer at 70°C rather than at room temperature, prior to centrifugation will give a modest increase in DNA yield per elution. Yields may further be increased by eluting a third time with the same 400 µl containing DNA.

Vacuum/Spin Protocol for Yeast DNA Isolation (V-Spin column only)(#D3570)

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

1. Prepare samples by following the standard Protocol in previous sections (Steps 1-7).
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 solution to the column.
4. Switch on vacuum source to draw the sample through the column, then turn off the vacuum.
5. Wash the column by adding 750 µl DNA wash buffer, draw the wash buffer through the column by turning on the vacuum source. Repeat this step with another 750 µl DNA wash buffer.
6. Assemble the column into a 2 ml collection tube and transfer the column to a micro centrifuge. Spin 1 minute to dry the column.
7. Place the column in a clean 1.5 ml microcentrifuge tube and add 100-200µl TE or water. Stand for 1-2 minute and centrifuge 1 minute to elute DNA.

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:

$$[DNA] = (Absorbance_{260}) \times (0.05\ \mu g/\mu l) \times (Dilution\ factor)$$

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 -20ºC to prevent degradation.

Troubleshooting

Problem	Possible Cause	Suggestions
Clogged Column	Incomplete lysis	Add the correct volume of Buffer YDL and incubate at 70°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} 10 per spin column. For larger volumes, divide sample into multiple tubes.
	Incomplete spheroblasting	Add 200 units of zymolase (lyticase) per 3 ml culture and incubate at 30°C for the specified time. It may be necessary to increase incubation by 15 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 70°C for 5 min with dH_2O or Tris buffer may increase yields.
	Improper washing	DNA Wash Buffer Concentrate must be diluted with absolute (100%) ethanol as specified on page 3 before use.
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.
	Poor cell lysis due to incomplete mixing with Buffer YDL	Repeat the procedure, this time making sure to vortex the sample with Buffer YDL immediately and completely.
	Incomplete cell lysis or protein degradation due to insufficient incubation.	Increase incubation time with Buffer YDL. Ensure that no visible cell clumps remain.
	Trace protein contaminants remain.	Following step 8, wash column with 300 µl Buffer YDL before proceeding to step 9.
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 200 units of zymolase (lyticase) per 3 ml culture and incubate at 30°C for the specified time. It may be necessary to increase incubation by 15 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 Concentrate.	Dilute Wash Buffer with the indicated volume of absolute ethanol before use.

Ordering Information

Product No.	Product Name	Description
Plant DNA and Plant RNA Isolation Kits		
D3485-01/02 D3486-01/02	Plant DNA Miniprep Kit	Isolation of total cellular DNA from dry and wet plant samples
D3487-01/02	Plant DNA Midiprep Kit	Isolation of total cellular DNA from up to 500 mg plant samples
D3488-01/02	Plant DNA Maxiprep Kit	Isolation of total cellular DNA from up to 2 gram dry and wet plant samples
R6627-01/02 R6827-01/02	Plant RNA Kit	Isolate total cellular RNA from plant samples
R6628-01/02	Plant RNA Midiprep Kit	Isolate up to 800ug total cellular RNA from 800plant samples
Fungal DNA and Fungal RNA Kit		
D3490-01/02 D3390-01/02	Fungal DNA Miniprep Kit	Isolation of total cellular DNA from dry and wet fungal samples
D3590-01/02	Fungal DNA Midiprep Kit	Isolation of total cellular DNA from up to 500 mg fungal samples
R6640-01/02 R6840-01/02	Fungal RNA Kit	Isolate total cellular RNA from fungal samples
Yeast, Bacterial, Mollusc DNA Kits		
D3473-01/02	Mollusc DNA Kit	Isolate genomic DNA from mollusc, arthropods, worms
D3450-01/02	Bacterial DNA Kit	Isolation genomic DNA from bacterial.
D3476-01/02	Yeast Plasmid Kit	Isolate yeast plasmid.