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Introduction

The E.Z.N.A.™ family of products is an innovative system that radically simplifies extraction and purification of nucleic acids from a variety of sources. Key to the system is Omega Bio-Tek's proprietary HiBind® matrix that avidly, but reversibly, binds DNA or RNA under certain optimal conditions allowing proteins and other contaminants to be removed. Nucleic acids are easily eluted with deionized water or low salt buffer.

The Yeast Plasmid Mini Kit combines the power of HiBind® technology with the alkaline-SDS lysis of yeast cells to deliver high quality DNA in less than 1 hour. Omega Bio-Tek's mini spin-columns facilitate the binding, washing, and elution steps, thus enabling multiple samples to be simultaneously processed. Yields vary according to plasmid copy number, yeast strain, and conditions of growth. Because yeast normally has very low copy number of plasmid, the maximum yield from 5 mL culture is around 1 ug.

This protocol has been successfully used to isolate autonomous plasmids from *S. cerevisiae*. As a modified alkaline lysis procedure, genomic DNA is virtually eliminated from the preparation. Note that all centrifugation steps are to be performed at room temperature.

Storage and Stability

All E.Z.N.A.™ Yeast Plasmid Mini Kit components are guaranteed for at least 12 months from the date of purchase when stored as follows: YP I/RNase A at 4°C, Lyticase at -20°C, all other material at 22-25°C.

Binding Capacity

Each HiBind® DNA Mini column can bind approximately 35 µg Plasmid DNA.

Kit Contents

Product Number	D3476-00 D3376-00	D3476-01 D3376-01	D3476-02 D3376-02
Purification Times	5 Preps	50 Preps	200 Preps
HiBind® DNA Mini Columns	5	50	200
2 mL Collection Tubes	5	50	200
Buffer YP I	5 mL	20 mL	60 mL
Buffer YP II	5 mL	20 mL	60 mL
Buffer YP III	5 mL	25 mL	80 mL
Equilibration Buffer	1.5 mL	7 mL	25 mL
Buffer SE	3 mL	30mL	110mL
Buffer HB	5 mL	30 mL	110 mL
DNA Wash Buffer Concentrate	1.5 mL	15 mL	3 x 25 mL
Glass beads	270 mg	2.7 g	10 g
Lyticase (units)	1,100	11,000	44,000
RNase A	50 µL	100 µL	400 µL
Elution Buffer	3 mL	10 mL	20 mL
Instruction Booklet	1	1	1

Before Starting

Briefly examine this booklet and become familiar with each step. Prepare all components and have the necessary materials ready before starting.

- Add vial of RNase A to bottle of YP I and Store at 4°C.

IMPORTANT	Dilute DNA Wash Buffer with absolute ethanol as follows	
	D3476-00 / D3376-00	Add 6mL absolute ethanol
	D3476-01 / D3376-01	Add 60 mL absolute ethanol
	D3476-02 / D3376-02	Add 100mL absolute ethanol per bottle
Prepare a lyticase stock solution at 5000 Unit/ml and aliquot into adequate portions. Store each aliquot at -20°C and thaw before use. Each sample will require 40 µl of this solution.		
D3476/D3376-00	Dissolve with 220µl of Buffer SE	
D3476/D3376-01	Dissolve with 2.2ml of Buffer SE	
D3476/D3376-02	Dissolve with 8.8ml of Buffer SE	

E. Z. N.A.™ Yeast Plasmid Mini Spin Protocol

Materials to Be Provided by User

- Tabletop micro-centrifuge and nuclease-free 1.5 mL tubes
- Water bath set to 30°C
- Absolute ethanol (96%-100%) - Do not use other alcohols
- 2-mercaptoethanol (β-mercaptoethanol)

1. Inoculate 5 mL YDP medium placed in a 10-20 mL culture tube with yeast carrying desired plasmid and grow at 30°C with agitation for 16-24 h.
2. Pellet 1-3 mL yeast culture (use 2×10^7 cells) by centrifugation at 5,000 × g for 5 min at room temperature.
3. **Discard medium and resuspend cells in 480 µL Buffer SE/2-mercaptoethanol and 40 µL lyticase solution.** Resuspend the pellet by vortexing at maxi speed for 1 minute. Complete resuspension of cell pellet is vital of obtaining good yields. Incubate at 30°C for at least 30 min.
Note: Remember to add 10 µl of 2-mercaptoethanol per 1 ml of Buffer SE before use. This mixture can be made and stored at room temperature for 1 week.
4. Pellet spheroblasts by centrifuging at 4,000 x g for 5 min at room temperature. Discard the supernatant completely.
5. **Resuspend the spheroblasts pellet with 250 µL Buffer YP I.**
6. **Add 50mg glass beads and vortex at max speed for 5 minutes.** Let stand to allow the beads to settle. Transfer the supernatant to a new 1.5 mL centrifuge tube (not supplied).
7. **Add 250 µL YP II and gently mix by inverting and rotating tube 4-6 times to obtain a cleared lysate.** A 5 min incubation at room temperature may be necessary. Avoid vigorous mixing as this will shear chromosomal DNA and lower plasmid purity. (Store YDP II tightly capped when not in use.)
8. **Add 350 µL YP III and gently mix by inverting several times until a flocculent white precipitate forms.** Centrifuge at $\geq 10,000 \times g$ for 10 minutes at room temperature.
9. **Prepare the column by adding 100 µl of Equilibration Buffer placed in a 2 mL collection tube. Centrifuge at $\geq 13,000 \times g$ for 30-60 seconds.** Discard the flow-through liquid

10. **CAREFULLY aspirate and add the clear supernatant to a clean HiBind® DNA mini column assembled in a 2 mL collection tube (provided).** Ensure that the pellet is not disturbed and that no cellular debris is carried over into the column. Centrifuge for 1 min at 10,000 × g at room temperature to completely pass lysate through column.
11. Discard flow-through liquid and wash the column by adding 500 µL of Buffer HB. Centrifuge for 1 min at 10,000 × g as above.
12. Discard flow-through liquid and wash the column by adding 700 µL of DNA Wash Buffer diluted with ethanol. Centrifuge for 1 min at 10,000 × g as above and discard flow-through.
NOTE: DNA Wash Buffer is supplied as a concentrate and must be diluted with absolute ethanol according to the instructions one bottle or on Page 3 under “**Before Starting.**”
13. **Optional Step:** Repeat wash step with another 700 µL DNA Wash Buffer.
14. Centrifuge the empty column for 2 min at maximal speed (≥13,000 × g) to dry the column matrix. **Do not skip this step - it is critical for removing ethanol from the column.**
15. **Place column into a clean 1.5 mL microcentrifuge tube. Add 50 µL to 100 µL (depending on desired concentration of final product) Elution Buffer (10mM Tris, pH8.5) directly onto the column matrix and centrifuge for 1 min at maximal speed (≥13,000 × g) to elute DNA.** This represents approximately 75-80% of bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration.
16. **Yield and quality of DNA:** Determine the absorbance of an appropriate dilution (20- to 50-fold) of the sample at 260 nm and then at 280 nm. The DNA concentration is calculated as follows:

$$\text{DNA concentration} = \text{Absorbance}_{260} \times 50 \times (\text{Dilution Factor}) \mu\text{g/mL}$$

Although the binding capacity of HiBind® DNA Column is around 30 µg, the yield of the yeast plasmid depends on the yeast strain and type of plasmid. High copy number plasmids generally yield up to 1 µg of DNA from 5 mL culture. The ratio of (absorbance₂₆₀)/(absorbance₂₈₀) is an indication of nucleic acid purity. A value greater than 1.8 indicates > 90% nucleic acid. Alternatively, quantity (as well as quality) can sometimes best be determined by agarose gel/ethidium bromide electrophoresis by comparison to DNA samples of known concentrations. Typically, the majority of the DNA eluted is in monomeric supercoil form, though concatamers may also be present.

E. Z. N.A.™ Yeast Plasmid Mini Vacuum/Spin Protocol

Carry out cell culture, lysis and neutralization as indicated in previous section (Steps 1-8). Instead of continuing with centrifugation, follow steps as below.

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

1. Prepare the vacuum manifold according to manufacturer’s instructions and connect the V-Spin column to the manifold.
2. Load the clear supernatant from Step 8 to the V-Spin column.
3. Switch on vacuum source to draw the sample through the column, then turn off the vacuum.
4. **Wash the column by adding 500 µL HB Buffer,** draw the wash buffer 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. (Optional) Repeat this step with another 700 µL DNA wash buffer.
6. Assemble the column into a 2 mL collection tube and transfer the column to a micro centrifuge. Spin at maximal speed (≥13,000 x g) for 2 minute to dry the column.
7. Place the column in a clean 1.5 mL microcentrifuge tube and add 30-50µL Elution Buffer (10mM Tris, pH8.5). Allow to stand for 1-2 minute and centrifuge at maximal speed (≥13,000 x g) for 1 minute to elute DNA.

Troubleshooting Guide

Problem	Likely Cause	Suggestions
Low DNA yields	Poor cell lysis	Do not use more than 5 mL (with high copy plasmids or 10 mL with low copy plasmids) culture with the basic protocol. Cells may not be dispersed adequately prior to addition of YP II. Vortex cell suspension to completely disperse. Increase incubation time with Solution II to obtain a clear lysate. YP II, if not tightly closed, may need to be replaced. Prepare as follows: 0.2 N NaOH, 1% SDS.
	Yeast culture overgrown or not fresh.	Do not incubate cultures for more than 24 hr at 30°C. Storage of cultures for extended periods prior to plasmid isolation is detrimental.
	Low copy-number plasmid used	Such plasmids may yield as little as 0.5µg DNA from a 5 mL overnight culture. Increase culture volume to 10 mL
No DNA eluted.	DNA Wash Buffer Concentrate not diluted with absolute ethanol.	Prepare DNA Wash Buffer Concentrate as instructed above.
High molecular weight DNA contamination of product.	Over mixing of cell lysate upon addition of YP II.	Do not vortex or mix aggressively after adding YP II. Adequate mixing is obtained by simply inverting and rotating tube to cover walls with viscous lysate.
Optical densities do not agree with DNA yield on agarose gel.	Trace contaminants eluted from column increase A ₂₆₀ .	Make sure to wash column as instructed in Steps 11 and 12. Alternatively, rely on agarose gel/ethidium bromide electrophoresis for quantization.
RNA visible on agarose gel.	RNase A not added to YP I.	Add 1 vial of RNase to each bottle of YP I.
Plasmid DNA floats out of well while loading agarose gel	Ethanol not completely removed from column following wash steps.	Centrifuge column as instructed in Step 13 to dry.

Ordering Information

Product No.	Product Name	Description
E.Z.N.A.™ Plasmid Mini System		
D6942-01/02 D6943-01/02	Plasmid Mini Kit I	Isolation of up to 30µg plasmid in 15 minutes
D6945-01/02	Plasmid Mini Kit II	Isolation of up to 70µg plasmid in 15 minutes
D7042-01/02 D7043-01/02	HP Plasmid Mini Kit I	Isolation of up to 30µg plasmid from end A+ bacterial in 25 minutes
D7045-01/02	HP Plasmid Mini Kit II	Isolation of up to 70µg plasmid from end A+ bacterial in 25 minutes
D6948-01/02	Endo-free Plasmid Kit I	Isolation of up to 30µg endotoxin free plasmid
D6950-01/02	Endo-free Plasmid Kit II	Isolation of up to 70µg endotoxin free plasmid
D3476-01/02 D3376-01/02	Yeast Plasmid Kit	Isolation of plasmid from yeast
D6900-01/02	M13 isolation kit	Isolation of M13 DNA from culture
E.Z.N.A.™ Plasmid Midi/Maxi Isolation System		
D6904-01/02	Plasmid Midi Kit	Isolation of ≥200µg plasmid with midi column
D6905-03/04	Fastfilter Plasmid Midi kit	Isolation of ≥200µg plasmid under 30 min
D6915-01/03/04	Endo-free Fastfilter Plasmid Midi kit	Isolation of up to 200µg endotoxin-free plasmid in less than 60 minutes
D6922-01/02	Plasmid Maxi Kit	Isolation ≥200µg plasmid with maxi column
D6924-01/03/04	Fastfilter Plasmid Maxi kit	Isolation of ≥1.5 mg plasmid under 30 min.
D6926-01/03/04	Endo-free Fastfilter Plasmid Maxiprep kit	Isolation of up to 1.5 mg endotoxin-free plasmid in less than 60 minutes
E-Z 96® Plasmid Isolation System		
D1097-01/02	E-Z 96® Fastfilter Plasmid Isolation Kit	Isolation of plasmid in 96 well format with lysate clearance plate
D1900-01	E-Z 96 M13 Isolation Kit	Isolation of M13 DNA in 96 well format