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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. Furthermore, the method can easily be adapted for plasmid isolation from *E.coli*. 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 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 Minicolumn	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
Buffer SE	3 ml	30 ml	110 ml
Buffer HB	5 ml	30 ml	110 ml
DNA Wash Buffer Concentrate	2 ml	20 ml	3 × 20 ml
Glass Beads	270 mg	2.7 g	10 g
Lyticase	250 units	2500 units	4x2500 units
RNase A	50 µl	100 µl	400 µl
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.

IMPORTANT

1. **Add vial of RNase A to bottle of YP I and store at 4°C.**
2. **DNA Wash Buffer Concentrate is to be diluted with absolute ethanol (96-100%) as follows:**

   D3476-00&D3376-00 Add 8 ml absolute ethanol

   D3476-01&D3376-01 Add 80 ml absolute ethanol

   D3476-02&D3376-02 Add 80 ml absolute ethanol/bottle

   Store diluted DNA Wash Buffer at room temperature !

3. **Prepare an Lyticase stock solution with Buffer SE at 2500units/ml and aliquot into portions.** Store each aliquot at -20°C and thaw before use. Each sample will require 20 µl this solution.

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

Materials to Be Provided by User

Have the following reagents and supplies ready:

- 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

1. **Inoculate 5 ml YPD medium placed in a 10-20 ml culture tube with Yeast carrying desired plasmid and grow at 30°C with agitation for 20-24 h.**
2. **Pellet 1-3 ml yeast culture (use < 2 x 10^7 cells) by centrifugation at 4,000×g for 5 min at room temperature.**
3. **Discard medium and resuspend cells in 480 µl Buffer SE, 10 µl 2-mercaptoethanol and 20 µl lyticase solution.** Resuspend the pellet by vortexing or pipetting. Complete resuspension of cell pellet is vital of obtaining good yields. Incubate at 30°C for at least 30 min.
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/RNase A.**
6. **Add 50mg glass beads and vortex at maxi speed for 5 minutes**. Let it 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 2 min incubation at room temperature may be necessary. Avoid vigorous mixing as this will shear chromosomal DNA and lower plasmid purity. (Store YP 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 ≥10,000 × g for 10 minutes at room temperature.
9. **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.

10. **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.

11. **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 on bottle or on Page 3 under "**Before Starting**."

12. **Optional Step: Repeat wash step with another 700 µl DNA Wash Buffer.**

13. **Centrifuge the empty column for 2 min at ≥13,000 × g to dry the column matrix.** Do not skip this step - it is *critical* for removing ethanol from the column.

14. **Place column into a clean 1.5 ml microcentrifuge tube. Add 30-50 µl (depending on desired concentration of final product) sterile deionized water (or TE buffer) directly onto the column matrix and centrifuge for 1 min at 10,000 ×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.

15. **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:

    DNA concentration = Absorbance$_{260}$ × 50 × (Dilution Factor) µg/ml
    Although the binding capacity of HiBind® DNA Column is around 35 ug, the yield of the yeast plasmid depends on the yeast strain and type of plasmid. High copy number plasmids generally yield up to 1µg of DNA from 5 ml culture. The ratio of (absorbance$_{260}$)/(absorbance$_{280}$) 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 Vacuum/Spin Protocol

(Product No. D3376 only, V-Spin column only)

Carry out cell culture, lysis and neutralization as indicated in previous section (Steps 1-8). Instead of continuing with centrifugation, follow steps 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 Buffer HB** , draw the wash buffer through the column by turning on the vacuum source.

5. **Wash the column by adding 700 µl DNA wash Buffer diluted with absolute ethanol.** Draw the wash buffer through the column by turning on the vacuum source. 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 microcentrifuge. Spin for 2 minute at maximum speed (≥13,000xg) to dry the column.

7. **Place the column in a clean 1.5 ml microcentrifuge tube and add 30-50µl TE Buffer or water.** Allow to stand for 1-2 minute and centrifuge for 1 minute to elute DNA.

Trouble Shooting Guide

Problem	Likely Cause	Suggestions
Low DNA yields	Poor cell lysis	Do not use more than 5 ml (with high copy number plasmids or 10 ml with low copy number 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 YP 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 16 hr at 37°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.	Wash Buffer Concentrate not diluted with absolute ethanol.	Prepare 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 10,11 and 12. Alternatively, rely on agarose gel/ethidium bromide electrophoresis for quantitation.
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	Applications	Cat. No.
Standard E.Z.N.A.™ Plasmid Isolation System		
Plasmid Mini Kit I	Isolation of up to 30 µg Plasmid DNA	D6942/3
Plasmid Mini Kit II	Isolation of up to 70 µg Plasmid DNA	D6945
Plasmid Midi Kit	Isolation of up to 250 µg Plasmid DNA	D6904
Plasmid Maxip Kit	Isolation of up to 1.5 mg Plasmid DNA	D6922
Fastfilter Plasmid Midi Kit	Isolation of up to 250 µg Plasmid DNA, featuring filter syringes for lysate clearance	D6905
Fastfilter Plasmid Maxi Kit	Isolation of up to 1.5 mg Plasmid DNA, featuring filter syringes for lysate clearance	D6924
E-Z 96 Fastfilter Plasmid Kit	Isolation of Plasmid DNA using a 96-well format	D1097
E-Z 96 SE Plasmid Kit	Isolation of plasmid DNA using a single plate	D1095
Yeast Plasmid Isolation Kit	Isolation fo Yeast Plasmid DNA	D3476
E.Z.N.A.™ Endotoxin Free Plasmid Isolation System		
Endo-Free Plasmid Mini Kit I	Isolation of up to 30 µg Endotoxin free Plasmid	D6948
Endo-Free Plasmid Mini Kit II	Isolation of up to 70 µg Endotoxin free Plasmid	D6950
Endo-Free Plasmid Mid Kit	Isolation of up to 250 µg Endotoxin free Plasmid DNA, featuring filter syringes for lysate clearance	D6915
EndoFree Plasmid Maxi Kit	Isolation of up to 1.5 mg Endotoxin free Plasmid DNA, featuring filter syringes for lysate clearance	D6926
E.Z.N.A.™ H P Plasmid Isolation System		
HP Plasmid Mini Kit I	Isolation of up to 30 µg of High Purity Plasmid	D7042
HP Plasmid MidiKit	Isolation of up to 200 µg of High Purity Plasmid	D7004
HP Plasmid Maxi Kit	Isolation of up to 1.5 mg of High Purity Plasmid	D7022
E.Z.N.A.™ Single Strand Phage DNA Isolation Kits		
M13 Isolation Kit	Isolation of up to 15µg of single stranded phage	D6900
E-Z 96 M13 Isolation Kit	Isolation of up to 15µg of M-13 DNA using a 96-	D1900
E.Z.N.A.™ Large Construct DNA Isolation Kits		
BAC/PAC DNA Isolation Kit	Effective purification of BAC or PAC DNA	D2156
BAC/PAC DNA Isolation Kit	purification of BAC or PAC using a 96-well format	D1056