## Contents





## 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 the Omega Biotek'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 DNA Kit combines the power of HiBind™ technology with the alkaline-SDS lysis of yeast cells to deliver high quality DNA in under 1 hour. Omega Biotek's mini-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 maxium yield from 5 ml culture is around 1 ug.

This protocol has been successfully used to isolate autonomous plasmid s 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.*(see page 5). Before starting note that all centrifugation steps are to be performed at room temperature. If DNA Wash Buffer is stored at 4°C, bring to room temperature before use.

**Storage and Stability**: All E.Z.N.A.™ Yeast Plasmid DNA 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, all other material at 22-25°C.

## Kit Contents

| Product Number | D3476-00<br>D3376-00 | D3476-01<br>D3376-01 | D3476-02<br>D3376-02 |
|---|---|---|---|
| Purifications | 5 | 50 | 200 |
| HiBind™ Miniprep Columns (I) | 5 | 50 | 200 |
| 2 ml Collection Tubes | 5 | 50 | 200 |
| YP I | 5 ml | 20 ml | 60 ml |
| YP II | 5 ml | 20 ml | 60 ml |
| YP III | 5 ml | 20 ml | 80 ml |
| Buffer HB | 5 | 30 ml | 100 ml |
| Wash Buffer Concentrate | 12 ml | 40 ml | 3 x 40 ml |
| RNase A, Concentrate | 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.

**Supplied By User**:
Microcentrifuge capable of at least 10,000 x g.
Sterile 1.5 ml centrifuge tubes.
Sterile deionized water (or TE buffer)
Absolute (96%-100%) ethanol

**IMPORTANT**

1. Add vial of RNase A to bottle of YP I provided. Store at 4°C.

2. DNA Wash Buffer Concentrate is to be diluted with absolute ethanol as follows:

| | |
|---|---|
| D3376-00<br>D3376-00 | Add 18 ml 100% ethanol |
| D3476-01<br>D3376-01 | Add 60 ml 100% ethanol to each bottle |
| D3476-02<br>D3376-02 | Add 60 ml 100% ethanol |

***Store diluted DNA Wash Buffer at room temperature***

**Note: All steps must be carried out at room temperature.**



## E. Z. N.A.™ Yeast Plasmid Miniprep Protocol

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 12-16 h.

2. Pellet 1.5-5 ml bacteria by centrifugation at 12,000 x g for 1 min at room temperature.

3. Decant or aspirate medium and discard. To the yeast pellet add 250 µl YP I/RNase A. Resuspend cells completely by vortexing. Complete resuspension of cell pellet is vital for obtaining good yields.

   NOTE: Some experiments show that pre-treatment of zymolase can improve the result of plasmid isolation from yeast. So if there is problem to lysis the cell with YPII or have low yield, do the following steps before Step 3 in this protocol.

   -Prepare Buffer SE as follows:

   1 M Sorbitol

   100 mM EDTA

   14 mM β-mercaptoethanol

   -Resuspend cells in 500 µl Buffer SE and add 200 units of zymolase. Incubate at 30°C for at least 30 min.

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

4. **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.)

5. **Add 350 µl YP III and gently mix by inverting several times until a flocculent white precipitate forms.** Centrifuge at 10,000 xg for 10 minutes at room temperature.

6. **CAREFULLY aspirate and add the clear supernatant to a clean Type I HiBind™ miniprep column (white) 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

1 min at 10,000 x g at room temperature to completely pass lysate through column.

7. Discard flow-through liquid and wash the column by adding 500 µl of Buffer HB. Centrifuge 1 min at 10,000 x g as above.

8. Discard flow-through liquid and wash the column by adding 750 µl of Wash Buffer diluted with ethanol. Centrifuge 1 min at 10,000 x g as above and discard flow-through.
**Note:** Wash Buffer Concentrate must be diluted with absolute ethanol before use. See label for directions. If refrigerated, Wash Buffer must be brought to room temperature before use.

9. Optional step: repeat wash step with another 750 µl Wash Buffer.

10 Centrifuge the empty column for 1 min at 10,000 x g to dry the column matrix. **Do not skip this step - it is critical for removing ethanol from the column.**

**11. Place column into a clean 1.5 ml microcentrifuge tube. Add 50 µl to 100 µl (depending on desired concentration of final product) sterile deionized water (or TE buffer) directly onto the column matrix and centrifuge 1 min at 10,000 x 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.

**12. 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:

$$DNA\ concentration = Absorbance_{260} \times 50 \times (Dilution\ Factor)\ \mu g/ml$$

Although the binding capacity of HiBind DNA Column is around 25 ug, the yield of the yeast plasmid variable depend on the yeast strains and type of plasmid. High copy number plasmids generally yield up to 1 µg 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.



## Vacuum/Spin Protocol for Yeast Plasmid Extraction (V-Spin column only)

Carry out cell culture, lysis, neutralization, and loading onto HiBind® DNA column as indicated previous protocols (step 1-5). Instead of continuing with centrifugation, follow steps blow.
Note: Please read through previous section of this book 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 5 to the V-Spin column I (blue).

3. Switch on vacuum source to draw the sample through the column and turn off the vacuum.

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

5. Wash the column by adding 750 µl DNA wash buffer, draw the wash buffer through the column by turn 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 30-50µl TE or water. Stand for 1-2 minute and centrifuge 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 plasmids or 10 ml with low copy plasmids) culture with the basic protocol.<br><br>Cells may not be dispersed adequately prior to addition of Solution II. Vortex cell suspension to completely disperse.<br><br>Increase incubation time with Solution II to obtain a clear lysate.<br><br>TP 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 Solution 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 7 and 8. Alternatively, rely on agarose gel/ethidium bromide electrophoresis for quantitation. |
| RNA visible on agarose gel. | RNase A not added to Solution 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 9 to dry . |



## Ordering Information

| Product No. | Product Name | Description |
|---|---|---|
| **E.Z.N.A.® Plasmid Miniprep System** | | |
| **D6942-01/02<br>D6943-01/02** | **Plasmid Miniprep Kit I** | **Isolation of up to 30µg plasmid in 15 minutes** |
| **D6945-01/02<br>D6946-01/02** | **Plasmid Miniprep Kit II** | **Isolation of up to 70µg plasmid in 15 minutes** |
| **D7042-01/02<br>D7043-01/02** | **High Performace Plasmid Miniprep Kit I** | **Isolation of up to 30µg plasmid from end A+ bacterial in 25 minutes** |
| **D7045-01/02<br>D7046-01/02** | **High Performace Plasmid Miniprep Kit II** | **Isolation of up to 70µg plasmid from end A+ bacterial in 25 minutes** |
| **E.Z.N.A.® Plasmid Midi/Maxi Isolation System** | | |
| **D6904-01/02** | **Plasmid Midiprep Kit** | **Midipreps in spin column format. Yield up to 200µg plasmid** |
| **D6922-01/02** | **Plasmid Maxiprep Kit** | **Maxipreps in spin column format. Yield up to 1mg plasmid** |
| **D7004-01/02** | **High Performace Plasmid Miniprep Kit** | **Isolation of up to 200µg plasmid from end A+ bacterial strains.** |
| **E-Z 96® Plasmid Isolation System** | | |
| **D1096-01/02** | **96 well Plasmid Kit** | **Isolation of plasmid in 96 well format** |

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