# EasyPrep™ Yeast Plasmid Miniprep Manual

Catalog#: YD01-01, YD01-02

Bioland

**For research use only**

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## Table of Contents

## Introduction

EasyPrep™ yeast plasmid kit is designed for rapid and reliable isolation of high-quality plasmid DNA from yeast cultures. Utilizing the reversible nucleic acid-binding properties of our matrix, the plasmid DNA is bound to the matrix while proteins and other unwanted impurities are eliminated by wash buffer. Pure DNA is then eluted. Purified DNA can be directly used in downstream applications such as PCR, restriction digestion, and Southern Blot.

The Yeast Plasmid Mini Kit combines the power of spin column technology with the lyticase, glass beads and alkaline-SDS lysis of yeast cells to yield high quality plasmid DNA in less than 1 hour. The mini spin columns facilitate the binding, washing, and elution steps, thus enabling multiple samples to be processed simultaneously. The actual plasmid yields depend on copy numbers, yeast strain, and conditions of growth. Because of low copy numbers, the maximum yield from 5 mL yeast culture is around 1 µg.

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 should be carried out at room temperature.

## Storage and Stability

All EasyPrep™ Yeast Plasmid Mini Kit components are guaranteed for at least 12 months from the date of purchase when stored as follows: Buffer YP I/RNase A mix at 4°C, Lyticase at -20°C, all other components at room temperature.

## Capacity

The binding capacity for the mini column is 40 µg of plasmid DNA.



**Kit Contents**

| Catalog# | YD01-01 | YD01-02 |
|---|---|---|
| Preps | 50 | 250 |
| DNA Mini Columns | 50 | 250 |
| Buffer YPI | 15 mL | 70 mL |
| Buffer YPII | 15 mL | 70 mL |
| Buffer YPIII | 20 mL | 100 mL |
| Buffer SE | 30 mL | 135 mL |
| Buffer KB | 28 mL | 135 mL |
| DNA Wash Buffer | 12 mL | 50 mL |
| Glass beads | 2.7 g | 13 g |
| Lyticase (units) | 11,000 U | 55,000 U |
| RNase A | 50 µL | 210 µL |
| Elution Buffer | 10 mL | 30 mL |
| Manual | 1 | 1 |

## Materials to Be Provided by Users

- Tabletop micro-centrifuge and nuclease-free 1.5 mL tubes.
- Water bath set to 30 °C.
- Absolute ethanol (96%-100%).

## Before Starting

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

**Important**

Dilute **DNA Wash Buffer** with **absolute ethanol** as follows ：

- **YD1271-01:** Add 48 mL absolute ethanol
- **YD1271-02:** Add 200 mL absolute ethanol per bottle

Prepare a **lyticase** stock solution with Buffer SE and aliquot into adequate portions. Store each aliquot at -20 °C and thaw before use. Each sample will require 30 µL of this solution.

- **YD1271-01:** Dissolve with 1.6 mL Buffer SE
- **YD1271-02:** Dissolve with 8 mL Buffer SE

Add vial of RNase A to bottle of YPI and store at 4°C



## EasyPrep™ Yeast Plasmid Miniprep Spin Protocol

1. Inoculate **5 mL YDP medium** 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 x $10^7$ cells) by centrifugation at 5,000 × g for 5 min at room temperature.
3. Discard medium and resuspend cells in **480 µL Buffer SE** with **30 µL lyticase solution.** Resuspend the pellet by vortexing at maxi speed for 1 min. 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 YPI.**
6. Add **50 mg glass beads** and vortex at max speed for 5 min. Let the sample stand to allow the beads to settle. Transfer the supernatant to a new 1.5 mL centrifuge tube.
7. Add **250 µL Buffer YPII** and mix by inverting and rotating the tube 4-6 times to obtain a cleared lysate. Incubate at room temperature for 5 min. Avoid vigorous mixing as this will shear chromosomal DNA and lower plasmid purity. Store Buffer YPII tightly capped.
8. Add **350 µL Buffer YP III** and mix completely by sharp hand-shaking several times until a flocculent white precipitate forms. Centrifuge at 13,000 × g for 10 min at room temperature.
9. **Carefully** transfer the clear supernatant to a DNA mini column. Ensure that the pellet is not disturbed and that no cellular debris is carried over into the column. Centrifuge at **10,000 × g** for 30 seconds. Discard the flow-through and put the column back to the collection tube.
10. Add **300 µL Buffer KB.** Centrifuge at **10,000 × g** for 30 seconds. Discard the flow-through and put the column back to the collection tube.

11. Add **700 µL DNA Wash Buffer.** Centrifuge at **10,000 × g** for 1 min. Discard flow-through. 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.
12. **Optional**: Repeat step 11.
13. Centrifuge the empty column, **with the lid open**, for 2 min at **13,000 x g** to dry the matrix. This step removes residual ethanol from the matrix.
14. Place column into a clean 1.5 mL microcentrifuge tube. Add **50-100 µL Elution Buffer** (10 mM Tris-HCL, pH 8.5) to the column matrix, let it stand by for 1 min, centrifuge at **13,000 x g for 1 min to** elute DNA.
15. Optional: Add the eluted DNA back to the column for a second elution yields 20-30% of additional DNA. The first elution normally yields 60-70% of the DNA.
16. Yield and quality of DNA: Determine the absorbance of an appropriate dilution (20-50 folds) of the sample at 260 nm and 280 nm. The DNA concentration is calculated as follows:

**DNA concentration = $A_{260}$ × 50 × (Dilution Factor) µg/mL**



## EasyPrep™ 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.

**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 column to the manifold.
2. Load the clear supernatant from **Step 8** in page 5 to the column.
3. Switch on vacuum source to draw the sample through the column, and then turn off the vacuum.
4. Add **500 µL Buffer KB to the column,** 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.
6. **(Optional)** Repeat this step with another **700 µL DNA Wash Buffer.**
7. Assemble the column into a 2 mL collection tube and transfer the column to a microcentrifuge. Spin at maximal speed **(13,000 x g)** for 2 min to dry the column.
8. Place the column in a clean 1.5 mL microcentrifuge tube and add **50-100 µL Elution Buffer (10 mM Tris-HCL, pH 8.5).** Let the column stand by for 1 min at room temperature and centrifuge at **13,000 x g** for 1 min to elute DNA.

## Trouble Shooting Guide

| Problems | Possible cause | Suggestions |
|---|---|---|
| Low DNA yield | Poor cell lysis | Do not use more than 5 mL (with high copy plasmids or 10 mL with low copy plasmids) culture with the |
| | Cells may not be dispersed adequately | Completely disperse the cell suspension by vortexing after adding Buffer YPI. After adding Buffer YPII, mix completely to obtain a clear lysate. |
| | Buffer 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 | Increase culture volume to 10 mL and scale up buffer volume. |
| No DNA eluted | Extended centrifugation during elution step at higher than 13,000 x g. Matrix may be present in eluate and cause abnormal OD readings. | If the centrifugation speeds higher than specified, some matrix residues may be co-purified with the plasmid DNA, but it will not interfere with PCR or restriction digests. Centrifuge the samples at suggested speed. |
| | Incomplete mixing with Buffer YPI | Repeat the procedure, this time making sure to vortex the sample with Buffer YPIII immediately and completely. |
| No DNA eluted | Insufficient mixing with Buffer YPII | Increase incubation time with Buffer YPII. Ensure that no visible cell clumps remain. |
| | DNA Wash Buffer not diluted with absolute ethanol. | Prepare DNA Wash Buffer as instructed above. |
| High molecular weight DNA contamination of product | Over mixing of cell lysate upon addition of Buffer YPII. | Do not vortex or mix aggressively after adding Buffer YPII. 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.<br>Alternatively, rely on agarose gel/ethidium bromide electrophoresis for quantization. |
| RNA visible on agarose gel | RNase A not added to Buffer YPI. | Add RNase A to Buffer YPI. |
| Plasmid DNA floats out of well while loading agarose gel | Ethanol not completely removed from column following wash steps. | Centrifuge column as instructed. |

## Limited use and warranty

*This product is warranted to perform as described in its labeling and in Bioland's literature when used in accordance with instructions. No other warranties of any kind, express or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by Bioland. Bioland's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of Bioland, to replace the products, Bioland shall have no liability for any direct, indirect, consequential, or incidental damage arising out of the use, the results of use, or the inability to use it product.*


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