Ver:2504

BW-YD1271 EZgene™ Yeast Plasmid Miniprep Kit

Contents

Kit Contents

Catalog#	BW-YD1271-00	BW-YD1271-01	BW-YD1271-02
Preps	10	50	250
Mini Columns	10	50	250
2 mL Collection Tubes	10	50	250
Buffer YPI	3 mL	15 mL	70 mL
Buffer YPII	3 mL	15 mL	70 mL
Buffer YPIII	4 mL	20 mL	100 mL
Buffer SE	6 mL	30 mL	135 mL
Buffer KB	6 mL	28 mL	135 mL
DNA Wash Buffer*	3 mL	15 mL	3 x 24 mL
Glass Beads	10×50 mg	50×50 mg	250×50 mg
Lyticase solution	400 µL	2 mL	8 mL
RNase A (20 mg/mL)	26 µL	130 µL	650 µL
Elution Buffer	2 mL	10 mL	30 mL
User Manual	1	1	1

*Add 12 mL (BW-YD1271-00) or 60 mL (BW-YD1271-01) or 96 mL (BW-YD1271-02) or 96-100% ethanol to each DNA Wash Buffer bottle before use.

Introduction

EZgene™ Yeast Plasmid Miniprep 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 DNA 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 EZgene™ Yeast Plasmid Miniprep Kit combines the power of spin column technology with

the lyticase solution, glass beads and alkaline-SDS lysis of yeast cells to yield high quality plasmid DNA in less than 1 hour. The Mini 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. The binding capacity for the Mini Column is 40 µg of plasmid DNA.

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

Store Buffer YPI/RNase A mixture store at 4℃, Lyticase solution store at -20℃. All other materials can be stored at room temperature (15-25℃). The guaranteed shelf life is 12 months from the date of production.

Before Starting

Prepare all components and get all necessary materials ready by examining this user manual and become familiar with each step and pay special attention to the followings.

Important Notes

✪ RNase A: 20 mg/mL. It is stable for one year at room temperature (15-25℃). Spin down RNase A vial briefly before adding to Buffer YPI.

✪ Buffer YPI should be stored at 4℃ once RNase A is added.

✪ Add 12 mL (BW-YD1271-00) or 60 mL (BW-YD1271-01) or 96 mL (BW-YD1271-02) 96-100% ethanol to DNA Wash Buffer bottle before use.

Materials not Supplied

- ✪ Tabletop micro-centrifuge and nuclease-free 1.5 mL tubes.
- ✪ Water bath set to 30℃.
- ✪ Absolute ethanol (96%-100%).
- ✪ DTT (DL-Dithiothreitol)

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets.

EZgene™ Yeast Plasmid Miniprep Spin Protocol

1. Inoculate 5 mL YDP medium with yeast carrying desired plasmid and grow at 30℃ with agitation for 16-24 hours.
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℃ for at least 30 min.

   Note： Ensure that20 µLof DTT (2M)per1mLBufferLYbeforeuse. This mixture is good at room temperature for 1 month.
4. Pellet spheroplasts by centrifuging at 4,000 ×g for 5 min at room temperature. Discard the supernatant completely.
5. Resuspend the spheroplasts 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.

   Note: 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 form. Centrifuge at 13,000 ×g for 10 min at room temperature.
9. Insert a **Mini Column** into a **2 mL Collection Tube**. Carefully transfer the clear supernatant to a **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 s. Discard the flow-through and put the column back to the collection tube.
10. Add **500 µL Buffer KB.** Centrifuge at 10,000 ×g for 30 s. Discard the flow-through and put the column back to the collection tube.
11. Add **600 µL DNA Wash Buffer.** Centrifuge at 10,000 ×g for 1 min. 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.
12. **Optional**: Repeat step **11**.

13. Centrifuge the empty column, with the lid open, for 2 min at 13,000 ×g to dry the matrix.

    Note： 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** to the column matrix, let it stand by for 1 min at room temperature, centrifuge at 13,000 ×g for 1 min to elute DNA.

    Note： This represents approximately 75-80% of bound DNA. An optional second elution step will yield any residual DNA, though at a lower concentration.

15. 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:

    Concentration (µg/mL) =OD_{260}×50×dilution factor.

EZgene™ Yeast Plasmid Miniprep 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 Mini Column.
3. Switch on vacuum source to draw the sample through the Mini 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 **600 µL DNA Wash Buffer**. Draw the wash buffer through the Mini Column by turning on the vacuum source.
6. Optional: Repeat this step with another 600 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 ×g) for 2 min to dry the column.

8. Place the Mini Column in a clean **1.5 mL microcentrifuge** tube and add **50-100 µL Elution Buffer**. Let the column stand by for 1 min at room temperature and centrifuge at 13,000 ×g for 1 min to elute DNA.

Trouble Shooting Guide

Problems	Possible Reasons	Suggested Improvements
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 basic protocol.
	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 YPII, if not tightly closed, may need to be replaced.	Prepare as follows: 0.2 M NaOH, 1% SDS.
	Yeast culture overgrown or not fresh.	Do not incubate cultures for more than 24 h at 30 ℃. 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 ×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 YPI immediately and completely.
	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. 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 were 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 BEIWO'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 BEIWO. BEIWO's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of BIOMIGA, to replace the products, BEIWO 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.

For technical support or learn more product information, please contact us at 400-115-2855 or visit our website at www.beiwobiomedical.com