96 Well Yeast Plasmid Miniprep

Introduction

EZgene™ 96-well yeast plasmid kit is designed for rapid and reliable isolation of high-quality plasmid DNA from yeast cultures in 96-well format. 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 and alkaline-SDS lysis of yeast cells to yield high quality plasmid DNA in less than 90 minutes. 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 1 mL yeast culture is around 0.2 -1 µg.

Materials supplied by user:

- Robotic station if using automated isolation.
- Centrifuge with swing-bucket rotor (4,000 x g).
- 96-well 2.0 mL deep well plates.
- Vacuum pump capable of achieving 300-400 mbar.
- Standard vacuum manifold.
- Oven or incubator preset to 70°C.

Storage and Stability

All components are guaranteed for 24 months from the date of purchase. The Buffer YP I/RNase A should be stored at 4°C.

Kit components

Catalog#	YD1281-01	YD1281-02
96-well DNA Plate	4x96	20x96
Buffer YP I	120 mL	600 mL
Buffer YP II	120 mL	600 mL
Buffer YP III	150 mL	750 mL
Buffer SE	180 mL	900 mL
DNA Wash Buffer	3x50 mL	5 x 200 mL
Lyticase (units)	1500 U	7500 U
RNase A	400 µL	2000 µL
Elution Buffer	80 mL	500 mL

Before Starting

Exam this handbook and get familiar with each step. Prepare all components and have the necessary materials ready.

- Briefly spin down the RNase A vial and add the RNase A to Buffer YP I and store at 4°C.
- DNA Wash Buffer: Add 200 mL (YD1281-01) or 800 mL (YD1281-02) 96-100% ethanol to each bottle before use.
- Add 16 mL (YD1281-01) or 80 mL (YD1281-02) Buffer SE to Lyticase and dissolve before use.
- Add 1% (v/v) of β-mercaptoethanol to Buffer SE before use.

Vacuum Manifold Protocol

1. Inoculate 1.2 mL appropriate selective YDP medium with yeast carrying desired plasmid in a 96-well deep well plate and grow at 30 °C with agitation for 18-24 h.

2. Seal the plate with a sealing film and pellet the cells by centrifugation at 3,000 rpm for 10 minutes at room temperature.

3. Discard medium completely and resuspend cells in 450 µL Buffer SE with 30 µL lyticase solution. Seal the plate with a sealing film and resuspend by vortexing at maxi speed for 1 min. Complete resuspension of cell pellet is vital of obtaining good yields. Spin down briefly and incubate at 30 °C for at least 30 min.
Note: Add 1% (v/v) of β-mercaptoethanol to Buffer SE before use.

4. Pellet spheroblasts by centrifuging at 3,000 g for 10 minutes at room temperature. Discard the supernatant completely**.** Add 250 µL Buffer YP I to each well and mix thoroughly by pipeting or seal the plate with sealing film and resuspend by vortexing. Spin down briefly.

5. Add 250 µL Buffer YP II to each well. Seal the plate with a sealing film and mix well by shaking and rotating. Incubate at room temperature for 5 times. Spin down the plate briefly.
Note: It is critical to mix the sample well for optimized plasmid yield.

6. Add 350 µL Buffer YP III to each well and seal the plate with a sealing film. Mix well by sharp shaking and vortex for 2 seconds.

7. Centrifuge the plate at 3000 x g for 10-15 minutes and transfer the clear lysate to a DNA binding plate.

8. Place the DNA plate on top of a vacuum manifold. Apply vacuum till

all liquid passes through the DNA plate.

9. Add 300 µL of Buffer KB to each well and apply vacuum till all the liquid passes through the DNA plate. Turn off the vacuum..

10. Add 750 µL of DNA Wash Buffer to each well and turn on vacuum till all buffer passes through the plate. Turn off vacuum. Repeat once.

11. Discard the waste in the manifold and dry the DNA plate with maximum vacuum power for 20 minutes.

12. Remove the DNA plate from the manifold and tap the plate on a stack of absorbent paper towels. Remove any residual moisture from the tip ends of the DNA plate with clean paper towel.

13. Optional: Place the DNA plate into a vacuum oven preset at 70°C for 10 minutes.

14. Place the DNA plate back to the vacuum manifold and apply maximum vacuum for another 5 minutes.

15. Place an elution plate or UV transparent plate inside the manifold with a manifold adaptor and set the DNA plate on top of the manifold.

16. Add 100-150 µL Elution Buffer or sterile water to each well, let the plate stand for 2 minutes. Apply maximun vacuum for 5 – 10 minutes to elute the DNA. Turn off vacuum and ventilate the manifold slowly.

Note: The DNA yield depends on the elution volume. Use less elution buffer if higher DNA concentration is desired. The first elution normally yields about 70% of the DNA. Add the eluted DNA back to the DNA plate for another elution yields another 20% of the DNA bound.

Centrifuge Protocol

1. Inoculate 1.2 mL appropriate selective YDP medium with yeast carrying desired plasmid and grow at 30 °C with agitation for 18-24 hours.

2. Seal the plate with a sealing film and pellet the cells by centrifugation at 3,000 g for 10 minutes at room temperature.

3. Discard medium completely and resuspend cells in 450 µL Buffer SE with 30 µL lyticase solution. Seal the plate with a sealing film and resuspend by vortexing at maxi speed for 1 min. Complete resuspension of cell pellet is vital of obtaining good yields. Spin down briefly and incubate at 30 °C for at least 30 min.

Note: Add 1% (v/v) of β-mercaptoethanol to Buffer SE before use.

4. Pellet spheroblasts by centrifuging at 3,000 rpm for 10 minutes at room temperature. Discard the supernatant completely**.** Add 250 µL Buffer YP I to each well and mix thoroughly by pipeting or seal the plate with a sealing film and resuspend by vortexing. Spin down briefly.

5. Add 250 µL Buffer YP II to each well. Seal the plate with a sealing film and mix well by shaking and rotating. Incubate at room temperature for 5 times. Spin down the plate briefly.

Note: It is critical to mix the sample well for optimized plasmid yield.

6. Add 350 µL Buffer YP III to each well and seal the plate with a sealing film. Mix well by sharp shaking and vortex for 2 seconds.

7. Centrifuge the plate at 3,000 x g for 10-15 min. Transfer the clear lysate into each well of the 96-well DNA plate.

8. Place the DNA plate on top of a deep well plate in a swing-bucket rotor and centrifuge at 3000 x g for 5 minutes.

9. Discard the flow through liquid and reuse the deep well plate for next step.

10. Add 300 µL Buffer KB to each well and centrifuge at 3000 x g for 5 minutes. Discard the flow-through liquid and reuse the deep well plate for next step.

11. Add 750 µL DNA wash buffer to each well and centrifuge at 3000 x g for 5 minutes. Decant the flow through liquid in the deep well plate. Repeat once.

13. Place the DNA plate on top of the deep well plate and centrifuge at 3000 x g for 10-15 minutes.

14. Remove the DNA plate from the deep well plate and tap the plate on a stack of absorbent paper towels. Remove any residual moisture from the tip ends of the DNA plate with clean paper towels.

15. **Optional**: Place the DNA plate into a vacuum oven preset at 70°C for 10 minutes.

16. Place the DNA plate on top of an elution plate or UV transparent elution plate. Add 75-100 µL of elution buffer or sterile water to each well of the DNA plate. Let the plate stand for 2 minutes.

17. Centrifuge the plate at 3000 x g for 5 minutes to elute the DNA.

Note: The DNA recovery rate and concentration depend on the elution volume. The first elution normally yields about 70% of the DNA. Add the eluted DNA back to the DNA plate for another elution yields another 20% of the DNA bound.

Troubleshooting Guide

Problem	Likely Cause	Suggestions
Low DNA Yields	Poor cell lysis	Do not use more than 2 mL of overnight culture. Resuspend the cell pellet completely. Buffer YP II if not tightly capped, may lead to poor cell lysis. Prepare fresh Buffer YP II follows: 0.2 N NaOH, 1% SDS.
	Cell overgrown	Do not grow yeast culture for more than 24 hours.
	Culture not fresh	Use fresh culture and do not storage the culture at 4°C.
	Low copy number plasmid	Scale up culture volume and buffer volume accordingly.
No DNA eluted	Forget to add ethanol to the DNA wash buffer	Prepare the DNA wash buffer as instructed.
Chromosomal DNA contamination	Over mixing after adding Buffer YP II	Do not vortexing or vigorously mixing after Buffer YP II is added.
OD doesn't match the DNA yield on agarsoe gel	Trace ethanol contamination	Wash the plate as instructed.
DNA flow out of agarose gel during loading	Trace ethanol contamination	Wash the plate as instructed.
RNA visible on agarose gel	Forget to add the RNase A to Buffer YP I	Spin down the RNA vial dd to Buffer YP I before use.
Lysate clearance plate clogged	Lysate was not mixed well after adding Buffer YP III	Mix the lysate well by inverting the plate for 5 times and then vortex for 10 seconds.