EZgene™ 96-Well Plasmid Kit

Contents

Introduction

The EZgene™ 96-well plasmid kit provides an easy and fast method for isolating high quality plasmid DNA in a high through put format. The key to this system is Biomiga's ezbind matrix that avidly, but reversibly, binds DNA under optimized buffer condition while proteins and other unwanted contaminants are removed by wash buffer. High quality plasmid DNAs are eluted with TE buffer or deionized water. By using the 96-well kit, up to 96 samples can be simultaneously processed in less than 90 minutes. The lysate clearance plate obviates the time-consuming centrifugation step and increases the DNA recovery up to 20%.

Note: The yield of plasmid DNA depends on the origin of the replication and the size of the plasmid. The protocols are optimized for high copy number plasmid purification. For low copy number plasmids, both the culture volume and the buffer volume need to be scaled up 3 to 5 times. Please contact our customer service for further information and reference the table below for the commonly used plasmids,

Plasmid	Origin	High copy	Low copy
pACYC	P15A		10-12
pSC101	pSC101		5
pSuperCos	pMB1		10-20
pBR322	pMB1		15-20
pUC	Muted pMB1	500-700	
pGEM®	Muted pMB1	300-400	
pBluescript®	ColE1	300-500	

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 A1/RNase A should be stored at 4°C. Buffer B1 needs to be tightly capped to avoid oxidation.

Kit components

Cat#	PD1812-00	PD1812-01	PD1812-02
Components	1x96 Preps	4 x 96 Preps	20 x 96 Preps
DNA plate	1	4	20
Lysate clearance plate	1	4	20
Buffer A1	60 mL	120 mL	600 mL
Buffer B1	60 mL	120 mL	600 mL
Buffer N1	80 mL	160 mL	750 mL
DNA Wash Buffer	50mL	3 x 50mL	5x200 mL
Buffer KB	60 mL	80 mL	1000 mL
Elution buffer	30 mL	70 mL	500 mL
RNase A	200 µL	400 µL	2000 µL
Breathable films	1	4	20
Sealing Films	4	16	80

Caution: Buffer N1 and Buffer KB contain chaotropic salts, wear gloves and protective eyewear when handling.

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 A1.
- DNA Wash Buffer: Add 200 mL (PD1812-01) or 800 mL (PD1812-02) 96-100% ethanol to each bottle before use.

Vacuum Manifold Protocol

1. Culture and harvest bacterial cells: Inoculate 1.0-1.2 mL LB/antibiotics medium in a 96-well 2 mL culture block with *E.coli* carrying desired plasmid and grow at 37 °C for 18-20 hours.

2. Seal the plate with sealing film and pellet the bacterial by centrifugation at 1,500-2000 x g for 5 minutes in a swing-bucket rotor at room temperature.

3. Remove the sealing film and discard supernatant. Tapping the inverted plate on a stack of absorbent paper towels to remove excess medium. Resuspend the pellet in each well with 250 µL of Buffer A1/RNase A by pipeting or vortexing (seal the plate with a sealing film if vortexing). No cell clumps should be visible after resuspension.

4. Wipe off any residual liquid on top of the plate and add 250 µL Buffer B1 to each well and mix thoroughly by gentle shaking/ rotating for 2 minutes and incubate at room temperature for 3 minutes. The solution should become viscous and slightly clear. **Note**: Vigorous mixing shears the chromosomal DNA.

5. Add 350 µL Buffer N1 to each well. Wipe off any residual buffers on the top of the plate and seal the plate with a sealing film. Mix by inverting the plate 5-10 times and vortex for 5 seconds. The flocculent white precipitate should form.

6. Assemble the vacuum manifold: 1) Place the DNA plate into the plate holder inside the manifold; 2) place the lysate clearance plate on top of the manifold. (The DNA plate should be positioned under the lysate clearance plate).

7. Immediately transfer the lysate into the lysate clearance plate. Allow the lysate to stand for 10 minutes. The white precipitate should float to the top. Apply vacuum till all the lysate passes through the lysate clearance plate.

8. Turn off the vacuum and discard the lysate clearance plate. Carefully transfer the DNA plate that contains the cleared lysate to the top of the vacuum manifold and turn on the vacuum till all the lysate passes through the DNA plate.

9. Optional: Add 500 µL of Buffer KB to each well and apply vacuum till all the liquid passes through the DNA plate. Turn off the vacuum..
Note: Buffer KB is required when the plasmid is being isolated from *endA*+ strains such as TG1, JM110, and HB101. It is not necessary if the plasmid is being purified from *endA*- strains such as Top 10 or DH5a.

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

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

14. Place a DNA Elution plate inside the manifold with a manifold adaptor and set the DNA plate on top of the manifold.

15. Add 100-150 µL Elution Buffer (or sterile water to each well, let the plate stand for 2 minutes. Apply maximum vacuum for 5 – 10 minutes to elute the DNA. Turn off vacuum and ventilate the manifold slowly.
Note: For maximum yield, add the eluted DNA back to the wells for a 2nd elution. The first elution normally yields 60-70% of the DNA while the 2nd elution yields another 20-30% of the DNA bound to the membrane.

Centrifuge Protocol

1. Culture and harvest bacterial cells: Inoculate 1.0-1.2 mL LB/antibiotics medium in a 96-well 2 mL culture block with *E.coli* carrying desired plasmid and grow at 37 ºC for 18-20 hours.

2. Seal the plate with a sealing film and pellet the bacterial by centrifugation at 1,500-2000 x g for 5 minutes in a swing-bucket rotor at room temperature.

3. Remove the sealing film and discard supernatant. Tapping the inverted block firmly in a stacked absorbent paper towels to remove excess medium. Resuspend the pellet in each well with 250 µL of Buffer A1/RNase A by vortexing or pipeting. Complete resuspension is critical for optimized plasmid yields.

4. Add 250 µL Buffer B1 to each well and mix thoroughly by gentle shaking and rotating for 1 minute and incubate at room temperature for 4 minutes. The solution should become viscous and slightly clear. **Note**: Vigorous mixing will shear the chromosomal DNA.

5. Add 350 µL Buffer N1 to each well. Wipe off any buffer residues on the top of the plate and seal the plate with a sealing film. Mix by inverting the plate for 5 times and vortex for 2 seconds. The flocculent white precipitate should form.

6. Place a lysate clearance plate on top of a 2 mL deep well plate. Transfer the lysate into the lysate clearance plate and allow the lysate to sit for 10 minutes. White precipitate should float to the top at this point.

7. Place the clearance/deep well plates in a swing-bucket rotor and centrifuge at 2000 x g for 5 minutes. Discard the lysate clearance plate.

8. Place a DNA plate on top of a 96-well deep plate and transfer the cleared lysate into the DNA plate.

9. Centrifuge at 3000 x g for 5 minutes.

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

11. **Optional:** Add 500 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.
Note: Buffer KB is required when the plasmid is being isolated from *endA*+ strains such as TG1, JM110, and HB101. It is not necessary if the plasmid is being purified from *endA*- strains such as Top 10 or DH5a.

12. Add 750 µL DNA wash buffer to each well and centrifuge at 3000 x g for 10 minutes.

13. 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 towels.

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

15. Place the DNA plate on top of a 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.

16. 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. For maximum yields, add the eluted DNA back to the wells for a 2nd elution. The first elution normally yields 60-70% of the DNA while the 2nd elution yields another 20-30% of the DNA bound to the membrane.

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 B1 if not tightly capped, may lead to poor cell lysis. Prepare fresh buffer B1 follows: 0.2 N NaOH, 1% SDS.
	Bacterial overgrown	Do not grow Bacterial culture for more than 16 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 B1	Do not vortexing or vigorously mixing after buffer B1 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 A1	Spin down the RNA vial dd to buffer A1 before use.
Lysate clearance plate clogged	Lysate was not mixed well after adding buffer N1	Mix the lysate well by inverting the plate for 5 times and then vortex for 10 seconds.