Endofree 96 Well Plasmid Mini Kit

Introduction

The endo free EZgene™ 96-well plasmid kit provides an easy and fast method for isolating endofree high quality plasmid DNA in a high through put format. The key to this system is Biomiga's proprietary endo removal buffer system that avidly, but reversibly, binds DNA to ezbind matrix under optimized condition while proteins and other unwanted contaminants are removed by wash buffer. High quality plasmid DNAs are eluted with endofree water. By using the 96-well kit, 96 samples can be simultaneously processed in less than 90 minutes. The purified DNA is ready for transfection of endotoxin sensitive cell lines in high-through-put format.

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:

- Centrifuge with swing-bucket rotor (4,000 x g).
- Vacuum pump capable of achieving 300-400 mbar.
- Standard vacuum manifold.
- Oven or incubator preset to 70°C.
- 100% ethanol.

Storage and Stability

All components are guaranteed for 12 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

Components	PD1818-02	PD1818-01
DNA plate	20	4
Lysate clearance plate	20	4
Elution Plate	20	4
Buffer A1	600 mL	120 mL
Buffer B1	600 mL	120 mL
Buffer N3	300 mL	60 mL
Buffer RET	600 mL	120 mL
Buffer KB	1000 mL	210 mL
DNA Wash Buffer	5 x 200 mL	200mL
Endofree Elution Buffer	600 mL	120 mL
RNase A	2000 µL	400 µL
Breathable Films	20	4
Sealing films	80	16

Caution: Buffer N3 contains acetic acid. Buffer RET and Buffer KB contain a chaotropic salt, 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, mix well before use. Store at 4°C after use.
- DNA Wash Buffer: Add 800 mL 96-100% ethanol to each bottle before use.
- Warm up Buffer B1 at 50°C to dissolve if precipitation forms.

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 5 minutes. The solution should become viscous and slightly clear. **Note**: Vigorous mixing will shear the chromosomal DNA.

5. Add 125 µL Buffer N3 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 2 seconds. The flocculent white precipitate should form.

6. Assemble the vacuum manifold: 1) Place a 2 mL deep well plate in the holder inside the manifold; 2) place the lysate clearance plate on top of the manifold. Note: Make sure to adjust the positions of samples between the lysate clearance plate and the deep well collection 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. Add 250 µL of Buffer RET to each sample in the deep well plate. Mix well by shaking and rotating the plate for 10 times. Set the DNA plate on top of the manifold.

9. Transfer the lysate/Buffer RET mix to each well of the DNA plate and apply vacuum till all the liquid passes through the DNA plate.

10. Add 500 µL of Buffer KB to each well and turn on vacuum till all buffer passes through the plate.

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

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

14. Optional: Place the DNA plate into a vacuum oven preset at 70°C for 10 minutes. This step increases DNA yield and purity.

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

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

17. Add 100-150 µL Endofree Elution Buffer 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: The first elution normally yields 70% of the DNA. Add the eluted DNA back to the DNA plate for a second elution yields another 20% of the DNA.

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°C 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 125 µL Buffer N3 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.

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 3000 x g for 5 minutes. Discard the lysate clearance plate.

8. Add 250 µL of Buffer RET to each well of the deep well plate, seal the plate with a sealing film and mix well by rotating and shaking the plate for 10 times.

9. Transfer the lysate/Buffer RET mix to a DNA plate on top of a deep well plate and centrifuge at 3000 x g for 5 minutes.

10. Discard the flow-through liquid and process the remaining lysate/Buffer RET mix as described. Discard the flow through and reuse the deep well plate for next step.

11. 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.

12. Add 700 µL DNA wash buffer 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. Repeat once.

13. Centrifuge the plate at 3000 x g for 10 minutes. This step removes residual ethanol for optimized elution in next step.

14. Remove the DNA 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. This step increases DNA yield and purity.

16. Place the DNA plate on top of an elution plate or UV transparent elution plate. Add 100-150 µL of Endofree Elution Buffer 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. For maximum yields and concentration, add the eluted DNA back to the DNA plate for a second elution. The first elution yields approximately 70% of the DNA while the second elution yields another 20% of the DNA.

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.