Contents

Introduction

Key to the kit is our proprietary DNA binding systems that allow the highly efficient binding of DNA to our ezBind™ matrix while proteins and other impurities are removed by Wash Buffer. Nucleic acids are easily eluted with sterile water or Elution Buffer.

Unlike other kits in the markets, no chaotropic salts are contained in the buffer of our patented plasmid purification kit. The purified DNA is guanidine/anion exchange resin residues free.

Plasmid isolated with traditional protocol normally contains high level of endotoxins (Lipopolysaccharides or LPS). For transfection of endotoxin sensitive cell lines or microinjection, the endotoxins should be removed before the applications. The EZgene™ endofree system uses a specially formulated buffer that extracts the endotoxin from the plasmid DNA. Two rounds of extraction will reduce the endotoxin level to 0.1 EU (Endotoxin) per µg of plasmid DNA.

This kit is designed for fast and efficient purification of plasmid DNA from 15 to 50 mL of E. coli culture. The midi column has a plasmid DNA binding capacity of 250 µg.

The purified endofree DNA is ready for high performance of downstream applications such as transfection of endotoxin-sensitive cell lines, primary cultured cells or microinjection.

Important Notes

Plasmid Copy Numbers: 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 2 times. Please contact our customer service for further information and reference Table 1 for the commonly used plasmids,

Table 1 Commonly used plasmid and expected yield.

Plasmid	Origin	Copy Numbers	Expected Yield (µg per 50 mL)
pSC101	pSC101	5	5
pACYC	P15A	10-12	5-10
pSuperCos	pMB1	10-20	10-20
pBR322	pMB1	15-20	10-20
pGEM®	Muted pMB1	300-400	100-150
pBluescript®	ColE1	300-500	100-200
pUC	Muted pMB1	500-700	150-250

Host Strains: The strains used for propagating plasmid have significant influence on yield. Host strains such as Top 10 and DH5a yield high-quality plasmid DNA. *endA*+ strains such as JM101, JM110, HB101, TG1 and their derivatives, normally have low plasmid yield due to either endogenous endonucleases or high carbohydrates released during lysis. We recommend transform plasmid to an *endA*- strain if the yield is not satisfactory. For purifying plasmid DNA from *endA*+ strains (Table 2), we recommend use product PD1712.

Table2 endA strains of E. Coli.

EndA*- Strains of *E. Coli							
DH5α	DH1	DH21	JM106	JM109	SK2267	SRB	XLO
TOP10	DH10B	JM103	JM107	SK1590	MM294	Stbl2™	XL1-Blue
BJ5182	DH20	JM105	JM108	SK1592	Select96™	Stbl4™	XL10-Gold

EndA*+ Strains of *E. Coli							
C600	JM110	RR1	ABLE® C	CJ236	KW251	P2392	BL21(DE3)
HB101	TG1	TB1	ABLE® K	DH12S ™	LE392	PR700	BL21(DE3) pLysS
JM101	JM83	TKB1	HMS174	ES1301	M1061	Q358	BMH 71-18
All NM strains				All Y strains			

Optimal Cell Mass (OD_{600} x mL of Culture): This procedure is designed for isolating plasmid grown in standard LB medium (Luria Bertani) for 12 -16 hours to a density of OD_{600} 2.0 to 3.0. If rich mediums such as TB or 2xYT are used, make sure the cell density doesn't exceed 3.0 (OD_{600}). A high ratio of biomass over lysis buffers result in low DNA yield and purity. The midi I column has an optimal biomass of 100-200. For example, if the OD_{600} is 3.0, the optimal culture volume should be 15 to 50 mL.

Culture Volume: Use a flask or tube 4 times bigger in volume than the culture medium to secure optimal condition for bacteria growth. Don't exceed the maximum culture volume suggested in the protocol. Incomplete lysis due to over amount of bacterial culture results in lower yield and less purity.

Storage and Stability

Buffer A1 should be stored at 4°C once RNase A is added. All other materials can be stored at room temperature (22-25°C). 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 instruction booklet and become familiar with each step.

Important

- RNase A: It is stable for more than half a year when stored at room temperature. Spin down the RNase A vial briefly. Add the RNase A solution to Buffer A1 and mix well before use. Store at 4℃.
- **Buffer B1 precipitates below room temperature. It is critical to warm up the buffer at 50℃ to dissolve the precipitates before use.**
- **Buffer N3 may form precipitates below 10°C, warm up at 37°C to dissolve the precipitates before use.**
- **Keep the cap tightly closed for Buffer B1 after use.**
- Make sure the availability of centrifuge (13,000 rpm). Especially, after mixing the lysate with ethanol, the sample needs to be processed immediately by centrifugation.
- Please make sure the DNA column is tighten (using the plastic wrench) ,w hen add the lysate to DNA column.
- ***Carry out all centrifugations at room temperature.***

Materials supplied by users

- 100% ethanol
- High speed centrifuge
- 50 mL high speed centrifuge tubes
- 50 mL conical tubes
- Isopropanol if precipitate the plasmid DNA.

Kit Contents

Catalog #	PD2468-00	PD2468-01	PD2468-02
Preps	2	10	25
ezBind™ Columns	2	10	25
Filter Syringe	2	10	25
2.0 ml Microfuge tube	4	20	25
Plastic wrench	1	1	1
Buffer A1	6 mL	30 mL	70 mL
Buffer B1	6 mL	30 mL	70 mL
Buffer N3	3 mL	15 mL	30 mL
Buffer RET	12 mL	60 mL	135 mL
DNA Washing Buffer*	5 mL	24 mL	54mL
RNase A (20 mg/mL)	0.6 mg (30 µL)	3 mg (150 µL)	7 mg (350 µL)
Endofree Elution Buffer	4 mL	20 mL	60 mL
User Manual	1	1	1

*Add 20 mL (PD2468-00) or 96 mL (PD2468-01) or 216 mL (PD2468-02) 96-100% ethanol to each DNA Wash Buffer bottle before use.

Safety Information

- Buffer N3 contains acetic acid, wear gloves and protective eyewear when handling.
- Buffer N3 and RET contains chaotropic salts, which may form reactive compounds when combines with bleach. Do not add bleach or acidic solutions directly to the preparation waste.

EZgene™ EndoFree Express Plasmid Midiprep Spin Protocol

1. **Inoculate 15-50 mL LB containing appropriate antibiotic with 100 µl fresh starter culture. Grow at 37ºC for 14-16 h with vigorous shaking.**
   Note: The best way to prepare a starter culture: Inoculate a single colony from a freshly grown selective plate into 1 ml LB medium containing the appropriate antibiotic and grow at 37°C for 6 -8 h with vigorous shaking (~250 rpm). ***Warning:*** **Do not use more than 50 ml culture.** Need to scale up buffers if processing more than 100 mL culture.

2. **Harvest the bacterial by centrifugation at 5,000 g for 10 min at room temperature. Pour off the supernatant and blot the inverted tube on paper towels to remove residual medium.**
   Note: Complete removal of residue medium is critical for bacteria lysis in the next step.

3. **Add 2.5 mL Buffer A1 and completely resuspend bacterial pellet by vortexing or pipetting.** Ensure that RNase A has been added into Buffer A1 before use.
   Note: Complete resuspension is critical for optimal yield.

4. **Add 2.5 mL Buffer B1, mix thoroughly by inverting 10 times with gentle shaking. Incubate for 5 -10 min to obtain a slightly clear lysate.** Complete lysis is critical for DNA yield. The mixture of completely lysed bacteria looks transparent.
   Attention: Buffer B1 forms precipitation below room temperature, if solution becomes cloudy, warm up at 37ºC to dissolve before use.

5. **Add 1 mL Buffer N3, mix completely by inverting the tube 5 times and shaking for 5 times.** It is critical to mix the solution well, if the mixture still appears conglobated, brownish or viscous; more mix is required to completely neutralize the solution.

6. Two options for clearing the lysates:

   High speed centrifuge**:** Transfer the lysate to a high speed centrifuge tube and centrifuge at 13,000 rpm (14,000-18,000 g) for 15 min at room temperature! Transfer the cleared lysate to a 50 ml conical tube.

ezFilter syringe: Pour the lysate into the barrel of the filter syringe and set the syringe in a 50 mL conical tube. Incubate at room temperature for 10 min. The white precipitates should float to the top. Gently insert the plunger to expel the cleared lysate to the tube, stop when feel strong resistance, some of the lysate may remain in the flocculent precipitate.

Note: To avoid clog of the syringe: Use less than 50 mL of overnight culture and mix the lysate well after adding Buffer C1. Alternatively, transfer the lysate to another syringe filter. Additional syringe filter can be purchased from Biomiga separately.

7. **Add 1 volume of Buffer RET (For example, 5 mL of Buffer RET to 5 mL of clear lysate), and 3 mL of 100% ethonal. Mix well by sharp hand shaking for 5 times.**

8. Add the lysate/ethanol mixture into a DNA column set in a 50 mL conical tube. Use the plunger to expel the lysate through the column.

   Note:Please make sure the DNA column is tighten (using the plastic wrench) ,when add the lysate to DNA column.

9. Dissemble the membrane column from the DNA column **using the plastic wrench.**Take the plunger out of the DNA column.Assemble the DNA column with the same membrane column and add the remaining mixture.Expel the plunger until all of mixture goes through the DNA binding membrane.

   Note:Please make sure to dissemble the membrane column from the DNA column before taking the plunger out of the column.Otherwise,the pressure difference between the inside and outside of the column would break the membrane.

10. Add **10 mL DNA Wash Buffer** to the assembled DNA column (dissemble the membrane column from the DNA column before taking the plunger out),expel the buffer out with the plunger. Repeat step "10".

11. Use the plastic wrench (Provided) to detach the end component from the midiprep column and insert it into a 2.0 ml eppendorf tube.

12. Spin the column at 13,000- 15,000 rpm (Max speed) for 1 min. Decant the flow through and put the column back to the tube. Spin the column at

max speed for 2 min, and then air-dry the membrane column at room temperature for 3-10min.

13. Insert the membrane column into a new sterile 1.5mL or 2.0mL **Microfuge tube.** Add **500 µL Endofree Elution Buffer (Prewarm the Elution Buffer 60℃ increases the yield) to the center of the column and incubate for 1 min at room temperature. Elute the DNA by centrifugation at 12,000 rpm for 1 min. Then a**dd **300 µL Endofree Elution Buffer to the center of the column and incubate for 1 min at room temperature. Elute the DNA by centrifugation at 12,000 rpm for 1 min.**

14. Repeat step "13" to improve the recovery.

15. Optional: Add the eluted DNA back to the column for another elution. The first elution normally yields about 60-70% of the DNA and the second elution yields another 20-30% of the DNA.
Note: The DNA is ready for downstream applications such as cloning or transfection of HEK293 cells.

.

EndoFree Express Plasmid Midiprep Flow Chart

Lysis and neutralization

Clear Lysate through a Syringe filter

Bind DNA to the DNA column

Wash the column with DNA Wash Buffer by a plunger

Detach the column and insert to a 2.0 mL tube. Spin the column to dry and elute the DNA.

Trouble Shooting Guide

Problem	Possible Reason	Suggested Improvement
Low Yield	Poor Cell lysis.	• Resuspend pellet thoroughly by votexing and pipetting prior adding buffer B1. • Make fresh buffer B1 if the cap had not been closed tightly. (Buffer B1: 0.2M NaOH and 1%SDS).
Low Yield	Bacterial culture overgrown or not fresh.	Grow bacterial 12-16 hours. Spin down cultures and store the pellet at –20°C . if the culture is not purified the same day. Do not store culture at 4°C over night.
Low Yield	Low copy-number plasmid.	Increase culture volume Increase the volume of buffer A1, B1, N3 and 100% ethanol proportionally with the ratio of 1:1:1.2:1.2.
No DNA	Plasmid lost in Host *E.coli*	Prepare fresh culture.
Genomic DNA contamination	Over-time incubation after adding buffer B1.	Do not vortex or mix aggressively after adding buffer B1. Do not incubate more than 5 minutes after adding solution B1.
RNA contamination	RNase A not added to solution A1.	Add RNase A to buffer A1.
Plasmid DNA floats out of wells while running in agarose gel, DNA doesn't freeze or smell of ethanol	Ethanol traces not completely removed from column.	Make sure that no ethanol residual remaining in the silicon membrane before eluting the plasmid DNA. Re-centrifuge or vacuum again if necessary.

Limited Use and Warranty

This product is intended for in vitro research use only. Not for use in human.
This product is warranted to perform as described in its labeling and in Biomiga'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 Biomiga. Biomiga's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of Biomiga, to replace the products, Biomiga 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 technology support or learn more product information, please visit our website at www.biomiga.com or contact us at (858) 603-3219.