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Introduction

Key to the kit is our proprietary DNA binding systems that allow the high efficient reversible binding of DNA to the mini column while proteins and other impurities are removed by wash buffer. Nucleic acids are then eluted with sterile water or elution buffer.

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. The endofree plasmid miniprep kit provides an efficient endotoxin removal step into the traditional purification procedure to produce transfection grade plasmid DNA.

This kit is designed for fast and efficient purification of plasmid DNA from 3 to 12 mL of *E. coli* culture. The mini column II has a DNA binding capacity of 80 μg.

The purified endofree DNA is ready for 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 reference Table 1 for the commonly used plasmids.

Table 1 Commonly used plasmid and expected yield.

Plasmid	Origin	Copy Numbers	Expected Yield (μg per 1 mL)
pSC101	pSC101	5	0.1-0.2
pACYC	P15A	10-12	0.4-0.6
pSuperCos	pMB1	10-20	0.4-1
pBR322	pMB1	15-20	0.6-1
pGEM®	Muted pMB1	300-400	6-7
pBluescript®	ColE1	300-500	6-8
pUC	Muted pMB1	500-700	8-12

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. Please reference Table 2 for the *endA* information.

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 medium 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 mini column II has an optimal biomass of 10-36. For example, if the OD_{600} is 3.0, the optimal culture volume should be 3-12 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℃ once RNase A is added. All other materials can be stored at room temperature (22-25℃). The guaranteed shelf life is 12 months from the date of purchase.

Before Starting

Two endotoxin removal procedures are provided. Protocol A removes endotoxin during the purification of plasmid DNA and Protocol B removes endotoxin after the purification of plasmid DNA.

Prepare all components and get all necessary materials ready by examining this instruction booklet and become familiar with each steps and pay special attention to the followings,

Important

- RNase A: It is stable for half a year under room temperature. Spin down RNase A vial briefly. Add the RNase A solution to buffer A1 and mix well before use.
- Add 8 mL (PD1222-00) or 60 mL (PD1222-01) or 96 mL (PD1222-02) 96-100% ethanol to each DNA Wash Buffer bottle before use.
- **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 ℃, warm up at 37 ℃ to dissolve the precipitates before use.**
- **Keep the cap tightly closed for Buffer B1 after use.**
- Ensure the availability of centrifuge capable of 13,000 rpm.
- ***Carry out all centrifugations at room temperature.***

Materials supplied by users

- 96-100% ethanol.
- 1.5 mL and 2.0 mL microcentrifuge tubes.
- High speed microcentrifuge or Vacuum manifold.

Kit Contents

Catalog #	PD1222-00	PD1222-01	PD1222-02
Preps	4	50	250
ezBind Columns	4	50	250
Buffer A1	2.5 mL	25 mL	125 mL
Buffer B1	2.5 mL	25 mL	125 mL
Buffer N3	400 µL	5 mL	25 mL
Buffer KB	2.5 mL	30 mL	135 mL
Buffer RET	4 mL	50 mL	250 mL
DNA Wash Buffer*	2 mL	15 mL	3 x 24 mL
Endofree Elution Buffer	1 mL	10 mL	30 mL
RNase A(20 mg/mL)	0.25 mg (17.5 µL)	2.5 mg (175 µL)	12.5 mg (625 µL)
User Manual	1	1	1

*Add 8 mL (PD1222-00) or 60 mL (PD1222-01) or 96 mL (PD1222-02) 96-100% ethanol to each DNA Wash Buffer bottle before use.

Safety Information

- Buffer N3 contain acetic acid, wear gloves and protective eyewear when handling.
- Buffer N3, KB 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 ™ Plasmid ezFlow Miniprep Spin Protocol:

1. Inoculate **3-12 mL** LB containing appropriate antibiotic with a single colony from a freshly streaked selective plate. Grow at 37℃ for 14-16 hours with vigorous shaking.

   Note: Do not use a streaked plate that has been stored at 4℃.
   Note: Do not inoculate culture directly with glycerol stock.
   Note: This protocol is optimized for *E. coli* strain cultured in LB medium. When using TB or 2xYT medium, special care needs to be taken to ensure the cell density doesn't exceed 3.0 (OD_{600}). Buffers need to be scaled up if over amount of cultures are being processed.

2. Harvest the bacterial culture by centrifugation for 1 minute at 13,000 rpm. Pour off the supernatant and blot the inverted tube on a paper towel to remove residue medium. Remove the residue medium completely.

   Note: Residue medium will cause,
   - Poor cell lysis and thus lower DNA yield.
   - Loose pellet after centrifugation in step 6.

3. Add **400 µL Buffer A1** and completely resuspend bacterial pellet by vortexing or pipetting. **(Complete resuspension is critical for bacterial lysis and lysate neutralization).**

4. Add **450 µL Buffer B1**, mix gently by inverting 10 times (do not vortex) and incubate at room temperature for 5 minutes.

   Note: Do not incubate for more than 5 minutes.

5. Add **80 µL Buffer N3**, mix completely by inverting/shaking the vial for 5 times.

   Note: It is critical to mix the solution well, if the mixture still appears conglobated, brownish or viscous; more mixing is required to completely neutralize the solution.

6. Centrifuge the lysate at 13,000 rpm for 10 minutes at room temperature.

   Note: If the lysate doesn't appear clean, reverse the tube angle, centrifuge for 5 more minutes and then transfer the clear lysate to DNA column.

7. Carefully transfer about **750 µL clear lysate（no more than 800 µL）** to a clean 2.0 mL tube and add **1 volume** of **Buffer RET (For example, 800**

µL of **Buffer RET to 800 µL** of **clear lysate)**, and **400 µL** of **100% ethanol**. Mix well by sharp hand shaking for 3 times.

8. Transfer **700 µL** of the **lysate/ethanol mixture** to a DNA spin column and centrifuge at 13,000 rpm for 20s. Discard the flow-through liquid and transfer the remaining **lysate/ethanol mixture** to the column. Centrifuge at 13,000 rpm for 30s and discard the flow-through, put the column back to the collection tube.

9. Add **500 µL Buffer KB** into the spin column, centrifuge at 13,000 rpm for 1 minute. Remove the spin column from the tube and discard the flow-through. Put the column back to the collection tube.

   Note: This step is important to remove residual protein contaminations especially for *end*A+ strains and be highly recommended for high qual i ty plasmid DNA.

10. Add **650 µL DNA Wash Buffer** and centrifuge at 13,000 rpm for 20s. Discard the flow-through liquid and insert the column, **with the lid open**, back to the collection tube. Repeat step "10".

11. Centrifuge the column, **with the lid open**, at 13,000 rpm for 5 -10 minutes to remove the residual ethanol.

    Note: Residual ethanol can be removed more efficiently with the column lid open. It is critical to remove residual ethanol completely.

12. Transfer the column to a 1.5 mL tube and add **50-100 µL** of **Endofree Elution Buffer** Incubate for 1 minute at room temperature and centrifuge at 13,000 rpm for 1 minute to elute DNA. Reload the eluate into the column (use the same 1.5 mL tube) and incubate for 1 minute, centrifuge at 13,000 rpm for 1 minute to elute DNA.

    Note: The eluted DNA is ready for transfection of endotoxin-sensitive cell lines, primary cultured cells or microinjection.

13. The DNA concentration can be calculated as follows,

    DNA concentration (µg/mL) = OD_{260nm} x 50 x dilution factor.

    Note: Two elutions give rise to maximum DNA yield. Use less Endofree Elution Buffer if high concentration is desired.

EZgene ™ Plasmid ezFlow Miniprep Spin/Vacuum Protocol:

1. Set up the vacuum manifold according to manufacture's instruction and connect the column to the manifold.

2. Carry out step 1-7 in previous protocol on page 6 and 7.

3. Carefully transfer the solution from step 7 in the previous protocol to a DNA column and turn on the vacuum to allow the lysate pass through the column. Repeat until the remaining **solution** pass through the column.

4. Add **500 µL Buffer KB** to the column and allow the buffer pass the column by vacuum.

   Note: This step is important to remove residual protein contaminations especially for *end*A+ strains and be highly recommended for high qual i ty plasmid DNA.

5. Add **650 µL** of **DNA Wash Buffer** to the column and allow the vacuum to draw the liquid through the manifold. Turn off the vacuum. Repeat step 5.

6. Transfer the column, **with the lid open**, to a 2 mL collection tube and centrifuge at 13,000 rpm for 5 - 10 minutes.

7. Transfer the column to an endofree 1.5 mL tube and add **50 -100 µL** of **EndoFree Elution Buffer**. Incubate for 1 minute and centrifuge at 13,000 rpm for 1 minute to elute DNA. Reload the eluate into the column (use the same 1.5 mL tube) and incubate for 1 minute, centrifuge at 13,000 rpm for 1 minute to elute DNA .

   Note: The eluted DNA is ready for transfection of endotoxin-sensitive cell lines, primary cultured cells or microinjection.

Purification of Low-Copy-Number Plasmid/Cosmid

The yield of low copy number plasmid is normally around 0.1 – 1 μg /mL of overnight culture. For isolating low copy number or medium copy number plasmid DNA, use the following guideline:

1. Culture volume: Use **2 x volumes** of the high copy number culture. Use 10 mL for miniprep kit.

2. Use **2 x volumes** of the **Buffer A1, Buffer B1, Buffer N3, Buffer RET** and **100% ethanol**. Additional buffers can be purchased from Biomiga.

3. Use **same volume** of **DNA Wash Buffer** and **Endofree Elution Buffer**.

Purification of plasmid > 12 kb

For isolating plasmid DNA > 12 kb, use the following guideline:

1. Culture volume: Use **2 x volumes** of the culture.

2. Use **2 x volumes** of the **Buffer A1**, **Buffer B1**, **Buffer N3, Buffer RET** and **100% ethanol**. Additional buffers can be purchased from Biomiga.

3. Use **same volume** of **Wash Buffer (DNA Wash Buffer)** and **EndoFree Elution Buffer**

4. Pre-warm the **Endofree Elution Buffer** at 65℃ and let the column stand for 5 min after adding **Endofree Elution Buffer.**

Trouble Shooting Guide

Problems	**Possible Reasons**	**Suggested Improvements**
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: 0.2 M 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℃ if the culture is not purified the same day. Do not store culture at 4℃ over night.
Low Yield	Low copy-number plasmid.	Increase culture volume according to instructions on page 9.
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 Buffer B1.
RNA contamination	RNase A not added to Buffer 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 elute the plasmid DNA. Re-centrifuge or vacuum again if necessary.
No phase partitioning after centrigugation	Temperature is lower than 23℃.	Make sure the temperature is greater than 23℃ for centrifugation or incubate the sample at 60℃ for 5 min and then perform centrifugation