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Introduction

Key to this kit is our proprietary DNA binding systems that allow the high efficient binding of DNA to our matrix while proteins and other impurities are removed by wash buffer. Nucleic acids are easily 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 miniprep column has a plasmid 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 contact our customer service for further information and reference the Table 1 for the commonly used plasmids,

Table 1 Commonly used plasmids.

Plasmid	Origin	Copy Numbers	Expected Yield (µg per 10 mL)
pSC101	pSC101	5	0.5-0.75
pACYC	P15A	10-12	1-1.5
pSuperCos	pMB1	10-20	1-2.5
pBR322	pMB1	15-20	1.5-2.5
pGEM ^R	Muted pMB1	300-400	30-40
pBluescript ^R	ColE1	300-500	30-50
pUC	Muted pMB1	500-700	50-70

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*.

<i>EndA</i>- Strains of <i>E. Coli</i>							
DH5 α	DH1	DH21	JM106	JM109	SK2267	SRB	XLO
TOP10	DH10B	JM103	JM107	SK1590	MM294	Stbl2 TM	XL1-Blue
BJ5182	DH20	JM105	JM108	SK1592	Select96 TM	Stbl4 TM	XL10-Gold
<i>EndA</i>⁺ Strains of <i>E. Coli</i>							
C600	JM110	RR1	ABLE [®] C	CJ236	KW251	P2392	BL21(DE3)
HB101	TG1	TB1	ABLE [®] K	DH12S TM	LE392	PR700	BL21(DE3) pLysS
JM101	JM83	TKB1	HMS174	ES1301	M1061	Q358	BMH 71-18
All NM strains				All Y strains			

Optimal Cell Mass (OD₆₀₀ 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 OD₆₀₀ 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 (OD₆₀₀). A high ratio of biomass over lysis buffers result in low DNA yield and purity. The mini column has an optimal biomass of 30-45. For example, if the OD₆₀₀ is 3.0, the optimal culture volume should be 10-15 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 purchase.

Before Starting

Alternative endotoxin removal procedures are provided. Protocol A removes endotoxin during the purification of plasmid DNA, while 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 of 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 (PD1212-00) or 60 mL (PD1212-01) or 96 mL (PD1212-02) 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 °C 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 pyrogen free microcentrifuge tubes
- High speed microcentrifuge
- Vacuum manifold if vacuum protocol is applied.

Kit Contents

Catalog #	PD1212-00	PD1212-01	PD1212-02
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	4 mL	30 mL	175 mL
Buffer KB	3 mL	30 mL	135 mL
DNA Wash Buffer*	2 mL	15 mL	3 x 24 mL
EndoClean Buffer	1 mL	10 mL	40 mL
Endofree Elution Buffer	1 mL	10 mL	30 mL
RNase A (20 mg/mL)	0.25 mg (12.5 µL)	2.5 mg (125 µL)	12.5 mg (625 µL)
User Manual	1	1	1

*Add 8 mL (PD1212-00) or 60 mL (PD1212-01) or 96 mL (PD1212-02) 96-100% ethanol to each DNA Wash Buffer bottle before use.

Safety Information

- Buffer N3 contains acidic acid, wear gloves and protective eyewear when handling.
- Buffer N3 and KB 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 Plasmid Miniprep Protocol **A**: Removal of Endotoxin *during* Plasmid Purification

This protocol is designed for removing the endotoxin during the plasmid purification.

A1. Spin Protocol

1. Inoculate **3-12 mL** LB containing appropriate antibiotic with a fresh colony. Grow at 37 °C for 14-16 hours with vigorous shaking.

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 (OD₆₀₀). Buffers need to be scaled up if over amount of cultures are being processed.

Note: Do not use a starter culture that has been stored at 4 °C.

Note: Do not grow starter culture directly from glycerol stock.

Note: The culture can be centrifuged at 6,000 rpm in a 15 mL conical tube for 10 minutes if high speed centrifuge tubes are not available. Alternatively, the cultures can also be spin down in multiple 2.0 mL tubes.

2. Harvest bacterial culture by centrifugation for 1 minute at 10,000 x g. Pour off the supernatant and blot the inverted tube on a paper towel to remove residue medium. Remove the residue medium completely.
3. Add **450 µL Buffer A1** and completely resuspend bacterial pellet by vortexing or pipetting (**Complete resuspension is critical for optimal yields**).
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.

Note: Buffer B1 precipitates (cloudy look) below room temperature. Warm up Buffer B1 at 50 °C to dissolve precipitation before use.

5. Add **100 µL Buffer N3**, mix completely by inverting/shaking the vial for 5 times and sharp hand shaking for 2 times.

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

6. Centrifuge the lysate at 12,000 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 the **clear lysate** into 2.0 mL tube and add **0.1 volume** of **EndoClean Buffer**. Mix by vortexing till homogeneous and incubate on ice for 10 minutes. Mix by tapping the tube several times during incubation.

Note: The solution becomes red and turbid after adding EndoClean Buffer. The solution becomes clear after incubation on ice.

Note: Use a serological pipet or a tip cut with a clean razor in the end to transfer the EndoClean Buffer.

Note: Mix the sample several times during incubation without leaving ice.

8. Centrifuge the solution at 12,000 rpm for 10 minutes at room temperature (the temperature must be greater than 23 °C). The solution should separate into 2 phases, the upper clear phase contains DNA and the lower organic phase contains endotoxin. The two phases will not separate if the temperature is less than 23 °C.

Note: If phase partitioning is not observed after centrifugation:

- Incubate the solution at 65 °C for 5 minutes. The solution becomes turbid again. And then repeat step 8.
- Or add **200 µL** Chloroform (37 °C), vortex to mix well, repeat step 8.

Note: Up to 99% of the endotoxin can be removed by extracting with the EndoClean Buffer once. Another extraction is necessary if less than 0.1 EU (Endotoxin)/ µg of DNA is desired by repeating step 7-8.

9. Transfer the clear phase lysate, avoid the colored phase, to a clean 2.0 mL. Add **450 µL** of **Buffer N3** and **400 µL** of **100% ethonal**. Mix well by sharp hand shaking for 3 times.
10. Transfer **700 µL** of the **lysate/ethanol mixture** to a DNA column and centrifuge at 13,000 rpm for 20s. Decant the flow-through liquid and insert the column back to the collection tube. Transfer the **remaining solution** to the column and centrifuge at 13,000 rpm for 20s. Decant the flow-through liquid and insert the column back to the collection tube.
11. **Optional:** 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: Buffer KB is recommended for *endA*⁺ strains such as HB101, JM101, TG1 or their derived strains. It is not necessary for isolating DNA from *endA*⁻ strains such as Top 10 and DH5a. Please reference Table 2 on page 3 for details.

12. Add **650 µL** of **DNA Wash Buffer** and centrifuge at 13,000 rpm for 20s. Decant the flow-through liquid and insert the column back to the collection tube. Repeat step “12”.
13. Centrifuge the column, **with the lid open**, at 13,000 rpm for 2 minute.

Note: It is critical to removes ethanol residues completely. The remaining ethanol will inhibit the elution of DNA from the column.

14. Transfer the column to an endofree 1.5 mL tube and add **50-100 µL** of **ddH₂O** or **Endofree Elution Buffer (Supplied)** . 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: If ddH₂O is applied, please make sure the pH is no less than 7.0 (7.0-8.5 is preferred). NaOH could be used to adjust the pH of ddH₂O.

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

15. Determination of DNA concentration,
Concentration (µg/mL) = OD_{260 nm} × 50 × dilution factor.

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

A2. 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 1-9 in previous protocol on page 6 and 7.
3. Carefully transfer the clear lysate from step 9 in the previous protocol to a DNA column and turn on the vacuum to allow the lysate pass through the column.
4. **Optional:** Add **500 µL Buffer KB** into the spin column and allow the buffer pass the column by vacuum.

Note: Buffer KB is recommended for *endA*⁺ strains such as HB101, JM101, TG1 or their derived strains. It is not necessary for isolating DNA from *endA*⁻ strains such as Top 10 and DH5a. Reference Table 2 on page 3.

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 2 minutes.
7. Transfer the column to an endofree 1.5 mL tube and add **50-100 µL of ddH₂O** or **Endofree Elution Buffer (Supplied)** . 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 DNA is ready for transfection of endotoxin-sensitive cell lines, primary cultured cells or microinjection.

EZgene™ EndoFree Plasmid Miniprep Protocol B: **Removal of Endotoxin *after* Plasmid Purification**

This protocol is designed for removing the endotoxin after the plasmid is purified.

1. Follow the protocol on Page 6 from Step 1 to 6.
2. Carefully transfer the clear lysate to a 1.5 mL tube and add **500 µL Buffer N3** and **400 µL 100% ethanol**. Mix well by sharp hand shaking for 2 times and go to step 10-15 on page 9-10.

Note: The plasmid DNA is purified. The following steps are for removal of endotoxin.

3. After the plasmid is purified, add **0.1 volume** of **EndoClean Buffer** to the plasmid sample in a 2 mL centrifuge tube (For example, add **10 µL EndoClean Buffer** to **100 µL plasmid sample**). The solution becomes turbid after adding EndoClean Buffer. If the temperature is below 20 °C, the solution remains clear.
4. Vortex the sample for 10s and incubate the tube on ice for 10 min. Mix the sample several times without leaving ice. The solution becomes clear after incubating on ice.
5. Centrifuge at 12,000 rpm at room temperature for 10 minutes (**the temperature must be greater than 23 °C for phase partitioning**). Carefully transfer the upper clear layer solution to another 2 mL tube.

Note: If phase partitioning is not observed after centrifugation, add **200 µL Chloroform**, vortex for 10s, and repeat step5.

6. Precipitate plasmid DNA with **0.1 volume** of **3 M KAc (pH 5.2)** and **0.7 volume of Isopropanol**. Centrifuge at 12,000 rpm for 10 min. Carefully decant the supernatant.
7. Add **1 mL 70% ethanol** and centrifuge at 12,000 rpm for 5 min.
8. Carefully decant and air-dry the DNA for 30 minutes in a hood. Resuspend the DNA with **ddH₂O** or **Endofree Elution Buffer (Supplied)** ..

Note: The DNA is ready for downstream applications such as cloning/subcloning, RFLP, Library screening, in vitro translation, sequencing, transfection, and 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 up to 25 mL for miniprep II.
2. Use **2 x volumes** of the **Buffer A1, Buffer B1, Buffer N3, and Buffer KB**. 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, and Buffer KB**. Additional buffers can be purchased from Biomiga.
3. Use **same volume** of **DNA Wash Buffer** and **Endofree Elution Buffer**.
4. Pre-warm the **Endofree Elution Buffer** at 65-70 °C and let the column stand for 5 mins after adding **Endofree Elution Buffer**.

Trouble Shooting Guide

Problems	Possible Reasons	Suggested Improvements
Low Yield	Poor Cell lysis.	- Resuspend pellet thoroughly by vortexing and pipetting prior adding Buffer B1. - Make fresh Buffer B1 if the cap had not been closed tightly. (Buffer B1: 0.2N 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 according to instructions on page 14.
No DNA	Plasmid lost in Host <i>E.coli</i>	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.

Related Products

Catalog #	Product Name	Preps	Price \$
PD1211-01	Plasmid mini kit	50	45.00
PD1211-02	Plasmid mini kit	250	220.00
PD1213-01	Plasmid mini kit II	50	60.00
PD1213-02	Plasmid mini kit II	250	250.00
PD1411-01	Plasmid midi kit	10	52.00
PD1411-02	Plasmid midi kit	25	125.00
PD1412-01	Plasmid midi kit II	10	79.00
PD1412-02	Plasmid midi kit II	25	175.00
PD1413-01	Plasmid ezFilter midi kit	10	79.00
PD1413-02	Plasmid ezFilter midi kit	25	175.00
PD1511-01	Plasmid maxi kit	10	120.00
PD1511-02	Plasmid maxi kit	25	250.00
PD1512-01	Plasmid ezFilter maxi kit	10	130.00
PD1512-02	Plasmid ezFilter maxi kit	25	280.00
PD1611-01	Plasmid ezFilter mega 3 kit	2	80.00
PD1611-02	Plasmid ezFilter mega 3 kit	10	380.00
PD1811-01	96-well plasmid mini kit	4x96	400.00
PD1811-02	96-well plasmid mini kit	20x96	2000.00