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Introduction

Key to the kit is our proprietary DNA binding systems that allow the high efficient binding of DNA to our ezBind™ matrix while proteins and other contaminates are removed under certain optimal conditions. Nucleic acids are easily eluted with sterile water or Elution Buffer.

This kit is designed for fast and efficient purification of plasmid DNA from 1 to 15 mL of E. coli culture. With the binding capacity of 80 μg, the yield obtained by Miniprep Kit II (PD1213) is higher than Miniprep Kit I (PD1211). The yield from 1 mL culture is typically around 8 to 12 μg.

The purified DNA is ready for downstream applications such as cloning/subcloning, RFLP, sequencing, and transfection of HEK293 cells.

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 3 to 5 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.

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 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. For over amount of cell numbers, either reduce the biomass or scale up the volumes of Buffer A1, B1 and N1.

Culture Volume: Use a flask or tube 4 times bigger in volumn thanthe 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

Prepare all components and get all necessary materials ready by examining this instruction booklet and become familiar with each steps.

Important

- RNase A: It is stable for more than half a year when stored at room temperature. Spin down RNase A vial briefly. Add the RNase A solution to buffer A1 and mix well before use. Store at 4℃.
- Add 8 mL (PD1213-00) or 60 mL (PD1213-01) or 96 mL (PD1213-02) or 60 mL (PD1213-03) 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.**
- 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, 2.0 mL microcentrifuge tubes.
- 15 mL conical tubes.
- High speed microcentrifuge or Vacuum manifold.

Kit Contents

Catalog#	PD1213-00	PD1213-01	PD1213-02	PD1213-03
Preps	4	50	250	100
ezBind Columns	4	50	250	100
Buffer A1	2.5 mL	25 mL	125 mL	50 mL
Buffer B1	2.5 mL	25 mL	125 mL	50 mL
Buffer N1	3 mL	30 mL	135 mL	60 mL
Buffer KB	3 mL	30 mL	135 mL	60 mL
DNA Wash Buffer*	2 mL	15 mL	3 x 24 mL	2 x 15 mL
Elution Buffer	1 mL	15 mL	60 mL	30 mL
RNase A (20 mg/mL)	0.25 mg (12.5 µL)	2.5 mg (125 µL)	12.5 mg (625 µL)	5 mg (250 µL)
User Manual	1	1	1	1

*Add 8 mL (PD1213-00) or 60 mL (PD1213-01) or 96 mL (PD1213-02) or 60 mL (PD1213-03) 96-100% ethanol to each DNA Wash Buffer bottle before use.

Safety Information

- Buffer N1 contains acidic acid, wear gloves and protective eyewear while handling.
- Buffer N1 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 ™ Plasmid Miniprep II Spin Protocol

For **1-4 mL** culture, reduce the volume of **Buffer A1, B1, N1** to **250 µL, 250 µL** and **350 µL**, respectively. And use the same volume of **DNA Wash Buffer** and **Elution Buffer**.

1. Inoculate **5-12 mL LB** containing appropriate antibiotic with a fresh colony. Incubate at 37 ℃ for 14-16 hours with vigorous shaking.

   Note: Prolonged incubation (> 16 hours) is not recommended since the *E.coli* starts to lyse and the plasmid yields may be reduced.
   Note: Do not grow the culture directly from the 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 (OD600). Buffers need to be scaled up proportionally if over amount of cultures are being processed.

2. Harvest bacterial culture by centrifugation for 1 min at 10,000 rpm. Pour off the supernatant and blot the inverted tube on a paper towel to remove residue medium. Remove the residue medium completely.

   Note: 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.

3. Add **450 µL Buffer A1** (Add RNase A to **Buffer A1** before use) and completely resuspend bacterial pellet by vortexing or pipetting.

   Note: 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. If necessary, continue inverting the tube until the solution becomes slightly clear.

   Note: Do not incubate for more than 5 minutes.
   Note: Buffer B1 precipitates (cloudy look) below room temperature. Warm up Buffer B1 at 37 ℃ to dissolve precipitation before use.

5. Add **550 µL Buffer N1**, mix completely by inverting/shaking the vial for 5 times and sharp hand shaking for 3 times.

   Note: Incubating the lysate in ice for 1 min will improve the yield.
   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 up to **700 µL** clear lysate into a DNA column with a collection tube, avoid the precipitations, spin at 13,000 rpm for 1 minute, discard the flow-through and put the column back to the collection tube. Carefully transfer the remaining clear lysate to the column and centrifuge at 13,000 rpm for 1 minute at room temperature and discard the flow-through in the collection tube. Put the column back to the collection tube.

8. **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.

9. Add **650 µL DNA Wash Buffer** *(Add ethanol to DNA wash buffer before use)* into the spin column, centrifuge at 13,000 rpm for 1 minute at room temperature. Remove the spin column from the tube and discard the flow-through. Repeat step "9" to improve the recovery.

10. Reinsert the spin column, **with the lid open**, into the collection tube and centrifuge for 2 minutes at 13,000 rpm.

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

11. Carefully transfer the spin column into a clean 1.5 mL tube and add **100-150 µL ddH_20** or **Elution Buffer** into the center of the column and let it stand for 2 minute. Elute the DNA by centrifugation at 13,000 rpm for 1 minute. Reload the eluate into the column and centrifuge again to improve the recovery.

    Note: The pH of Elution Buffer or ddH_2O will affect the plasmid DNA elution. If ddH_2O 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 ddH_2O.
    Note: The DNA is ready for downstream applications such as cloning/subcloning, RFLP, library screening, *in vitro* translation, sequencing, transfection of robust cells such as HEK293 cells.
    Note: It's highly recommended to remove the endotoxin (PD1214) if the DNA is used for endotoxin-sensitive cell lines, primary cultured cells or microinjection.

12. The DNA concentration can be calculated as,
    Concentration (µg/mL) = OD_{260} nm x 50 x dilution factor.

EZgene™ Plasmid Miniprep II 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 1-6 on Page 6 in previous protocol.

3. Carefully transfer the clear lysate to the 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 lysate pass through 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. Please 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. Carefully transfer the spin column into a clean 1.5 mL tube and add **100-150 µL dd$H_2$0** or **Elution Buffer** into the column and let it stand for 1 minute. Elute the DNA by centrifugation at 13,000 rpm for 1 minute. Reload the eluate into the column and centrifuge again to improve the recovery.

   Note: The DNA is ready for downstream applications such as cloning, RFLP, library screening, *in vitro* translation, sequencing and transfection of robust cells such as HEK293 cells.

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 25 mL for the miniprep II.

2. Use **2 x volumes** of the **Buffer A1**, **Buffer B1** and **Buffer N1**. Additional buffers can be purchased from Biomiga.

3. Use **same volume** of **Wash Buffer (DNA Wash Buffer)** and **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** and **Buffer N1**. Additional buffers can be purchased from Biomiga.

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

4. Pre-warm the **Elution Buffer** at 65-70℃ and let the column stand for 5 mins after adding **Elution Buffer.**

Trouble Shooting Guide

Problems	Possible Reasons	Suggested Improvements
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 and scale up the volume of buffers according to the instruction 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 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	Trace EtOH not completely removed from column.	Make sure that no ethanol residues remain in the silicon membrane before elute the plasmid DNA. Re-centrifuge or vacuum again if necessary.