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Introduction

Key to this kit is our proprietary DNA binding systems that allow DNA exclusively and efficiently bind 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, our patented plasmid purification kit has no chaotropic salts in the buffers, the purified DNA is guanidine/anion exchange resin residues free which enable the high performance of downstream applications.

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 yield from 50 mL culture is typically around 150 to 250 μg.

The purified DNA is ready for high performance of downstream applications such as transfection of robust cells such as HEK293, restriction mapping, library screening, sequencing, as well as gene therapy and genetic vaccinations.

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 Table1 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 column has an optimal biomass of 100-150. For example, if the OD_{600} is 3.0, the optimal culture volume should be 25-50 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 ℃ 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 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.**
- Keep the cap tightly closed for Buffer B1 after use.
- Make sure the availability of centrifuge, especially, after mixing the lysate with ethanol, the sample needs to be processed immediately either by centrifugation.
- ***Carry out all centrifugations at room temperature.***

Materials supplied by users

- 70% ethanol and 100% ethanol.
- High speed centrifuge.
- 30 mL high speed centrifuge tubes.
- 15 mL and 50 mL conical tubes.
- 1.5 mL tubes.
- Isopropanol if precipitate the plasmid DNA.

Kit Contents

Catalog	PD1411-00	PD1411-01	PD1411-02
Preps	2	10	25
ezBind™ Columns	2	10	25
Buffer A1	6 mL	30 mL	70 mL
Buffer B1	6 mL	30 mL	70 mL
Buffer C1	7 mL	35 mL	85 mL
Buffer KB	7 mL	35 mL	85 mL
RNase A (20 mg/mL)	0.6 mg (30 µL)	3 mg (150 µL)	7 mg (350 µL)
Elution Buffer	5 mL	25 mL	60 mL
User Manual	1	1	1

Safety Information

- Buffer C1 contains acidic acid, wear gloves and protective eyewear when handling.
- Buffer C1 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 Midiprep Spin Protocol

1. Inoculate **15-50 mL** LB containing appropriate antibiotic with 50 μL fresh starter culture. Incubate at 37 ℃ for 14-16 hours 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 ℃ for 8 hours with vigorous shaking (~250 rpm) and then use the culture as starter culture.
   Note: Do not use more than 50 mL culture or cell mass greater than 150. The buffer volume needs to be scaled up if processing over 100 mL of culture.
   Note: Do not use a starter culture that has been stored at 4 ℃.
   Note: Do not grow starter culture directly from glycerol stock.

2. Harvest the bacterial by centrifugation at 5,000 x g for 10 minutes at room temperature. Pour off the supernatant and blot the inverted tube on paper towels to remove residual medium.

3. Add **2.5 mL Buffer A1** (Add RNase A to **Buffer A1** before use) and completely resuspend bacterial pellet by vortexing or pipetting **(Complete resuspension is critical for optimal yields)**.

4. Add **2.5 mL Buffer B1,** mix gently but thoroughly by inverting 5 times and incubate for 5 minutes to obtain a slightly clear lysate.

   Note: Do not incubate longer than 5 min. Over-incubating causes genomic DNA contamination and plasmid damage.

5. Add **3.0 mL Buffer C1**, mix immediately by inverting 5 times and sharp hand shaking for 3 times.

   Note: Adding ice cold C1 or incubating the lysate in ice will decrease the floating precipitates at step "6".
   Note: 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. Transfer the lysate to a high speed centrifuge tube and centrifuge at 14,000 x g for 10 minutes at room temperature.

   Note: If the rotor is cold, incubate the lysate at room temperature for 10 minutes and then perform centrifugation as described.

7. Carefully transfer the clear supernatant into a 15 mL tube (avoid the floating precipitates). Add **3.0 mL 100% ethanol.** Mix immediately by sharp hand-

shaking. The mixture of ethanol/lysate needs to be centrifuged through to the DNA column immediately.

8. Immediately transfer **6.0 mL** the **lysate/ethonal mix** into a DNA column with a 15 mL collection tube. Centrifuge at > 2,500 x g for 1 minute at room temperature. Remove the column from the tube and discard the flow-through liquid. Reinsert the column to the collection tube. Repeat step "8" till all the lysate/ethonal mix has been passed through the column.

9. **Optional:** Add **3.0 mL Buffer KB** into the spin column, centrifuge at > 2,500 x g 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.

10. Add **4.0 mL 70% ethanol** into the column, centrifuge at > 2,500 x g for 1 min. Remove the column from the tube and discard the flow through. Reinsert the column into the collection tube. Repeat step "10".

11. Centrifuge the column, **with the lid open**, at > 2,500 x g for 10 min to remove the ethanol residues.

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

12. Carefully transfer the column into a sterile clean 15 mL tube and add **0.5 mL Elution Buffer or sterile ddH$_2$O** to the center of the column and incubate for 1 min at room temperature. Elute the DNA by centrifugation at > 2,500 x g for 5 min.

13. For higher yield, reload the eluate in the 15 mL tube to the column and incubate for 1 min. Elute the DNA again by centrifugation at > 2,500 x g for 5 min.

    Note: If ddH$_2$O 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$_2$O.
    Note: The DNA is ready for downstream applications such as cloning/subcloning, RFLP, Library screening, *in vitro* translation, sequencing, transfection of robust HEK293 cells.It's highly recommended to remove the endotoxin (PD1415) if the DNA is used for endotoxin-sensitive cell lines, primary cultured cells or microinjection.

    Note: The first elution normally yield 60-80% of the DNA bound. Two elutions increase the DNA recovery up to 90%. For maximum yield and higher concentration,

pool the elutions together, add 0.1 volume 3M NaAc and 0.7 volume isopropanol. Mix well by sharp hand shaking and centrifuge at top speed for 10 min. Remove the supernatant. Wash the DNA with 800 μL 70% ethanol, centrifuge for 5 min, carefully decant. Air-dry the pellet for 5-10 min. Resuspend the DNA in Elution Buffer or Sterile ddH_2O**.**

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

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 **100 mL** for midiprep.

2. Use **2 x volumes** of the **Buffer A1**, **Buffer B1** and **Buffer C1**. Additional buffers can be purchased from Biomiga.

3. Use **same volume** of **Wash Buffer (70% ethanol)** and **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: 0.2N 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 ℃. 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. Increase the volume of Buffer A1, B1, C1 and 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 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 eluting the plasmid DNA. Re-centrifuge or vacuum again if necessary.