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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 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 buffer. The purified DNA is guanidine/anion exchange resin residues free.

This kit is designed for fast and efficient purification of plasmid DNA from 100 to 250 mL of *E. coli* culture. The maxi column has a plasmid DNA binding capacity of 1 mg.

The purified plasmid DNA is ready for 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 to 3 times. Reference Table 1 for the commonly used plasmids,

Table 1 Commonly used plasmids.

Plasmid	Origin	Copy Numbers	Expected Yield (μg per 200 mL)
PSC101	pSC101	5	12
pACYC	P15A	10-12	25-40
pSuperCos	pMB1	10-20	30-50
PBR322	pMB1	15-20	35-50

pGEM®	Muted pMB1	300-400	350-450
pBluescript®	ColE1	300-500	450-600
PUC	Muted pMB1	500-700	700-1,000

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 PD1713.

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 maxi column has an optimal biomass of 450-550. For example, if the OD_{600} is 2.5, the optimal culture volume should be 200 mL.

Culture Volume: Use a flask or tube 4 times larger 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, and then 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.**
- Incubating Buffer C1 at 4 ^{o}C before experiment will decrease the floating precipitates.
- Keep the cap tightly closed for Buffer B1 after use.
- Make sure the availability of centrifuge and vacuum manifold, especially, after mixing the lysate with ethanol, the sample needs to be processed immediately either by centrifugation or vacuum.

- ***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
- 50 mL tubes

Kit Contents

Catalog#	**PD1512-00**	**PD1512-01**	**PD1512-02**
Preps	2	10	25
ezBind™ Columns	2	10	25
Filter syringe (60 mL)	2	10	25
Buffer A1	22 mL	110 mL	270 mL
Buffer B1	22 mL	110 mL	270 mL
Buffer C1	27 mL	135 mL	330 mL
RNase A (20 mg/mL)	2.2 mg (110 µL)	11 mg (550 µL)	27 mg (1.35 µL)
Elution Buffer	6 mL	30 mL	60 mL
User Manual	1	1	1

Safety Information

Buffer C1 contains acidic acid, wear gloves and protective eyewear when handling.

EZgene™ Plasmid Maxiprep Spin Protocol

1. Inoculate **150- 200 mL** LB containing appropriate antibiotic with 100 µL fresh starter culture. Incubate at 37 ℃ 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 ℃ for 6-8 h with vigorous shaking (~250 rpm).
   Note: Do not use more than 200 mL culture or cell mass greater than 550. The buffer volume needs to be scaled up if processing over 200 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 completely.

3. Add **10 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 **10 mL Buffer B1**, mix gently but thoroughly by inverting 5-10 times. If necessary, continue inverting the tube until the solution becomes slightly clear. Incubate at room temperature for 5 minutes to obtain a slightly clear lysate.

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

5. Add **2.8 mL Buffer C1**, mix immediately by inverting/shaking the vial for 5 times and sharp hand shaking for 5 times.

   Note: Adding ice cold C1 or incubating the lysate in ice will decrease the floating precipitates at step "6".

6. **Optional 1:** Transfer the lysate to a high speed centrifuge tube and centrifuge at 14,000 x g for 10 minutes at room temperature. Carefully transfer the clear supernatant into a 15 mL tube (avoid the floating precipitates).

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

   Optional 2: Pour the lysate directly into the barrel of the filter syringe. Insert the syringe to a clean 50 mL tube (not supplied) set in a rack. Allow the cell lysate to sit for 10 minutes. The white precipitates should float to the top. Hold the filter syringe barrel over the 15 mL tube and gently insert the plunger to expel the cleared lysate to the tube, stop when feel major resistance, some of the lysate may remain in the flocculent precipitate, **do not force the residual lysate through the filter.**

7. Carefully transfer the clear supernatant into a 50 mL tube (avoid the floating precipitates). Add **9.2 mL Buffer C1** and **12 mL 100% ethanol.** Mix immediately by sharp shaking. The mixture of ethanol/lysate needs to be centrifuged through the DNA column immediately.

8. Immediately apply **20 mL** of the **lysate/ethanol mixture** to a DNA column with the collection tube. Centrifuge at > 2,500 x g for 1 minute at room temperature. Discard the flow-through liquid and put the column back to the collection tube. Add the remaining **lysate/ethanol mixture** to the DNA column and centrifuge at 5,000 x g for 1 minutes. Discard the flow-through liquid and put the column back to the collection tube.

9. Add **10 mL 70% ethanol** into the column, centrifuge at > 2,500 x g for 1 minute. Remove the column from the tube and discard the flow through.

Reinsert the column into the collection tube. Repeat step 9.

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

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

11. Carefully transfer the column into a sterile clean 50 mL tube and add **1-1.5 mL Sterile ddH_2O or Elution Buffer** to the center of the column and incubate for 1 minute at room temperature. Elute the DNA by centrifugation at 5,000 x g for 5 minutes.

12. For higher yield, reload the eluate in the 50 mL tube to the center of the column and incubate for 1 minute at room temperature. Elute the DNA again by centrifugation at 5,000 x g for 5 minutes.

    Note: If ddH_2O is used for eluting DNA, make sure the pH is ≥ 7.0.

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

    Note: It's highly recommended to remove the endotoxin (PD1514) if the DNA is used for transfection of endotoxin-sensitive cell lines, primary cultured cells or microinjection.

    Note: Two elutions give rise to maximum DNA yield. For maximum yield and higher concentration, pool the elutions together, add 0.1 volume 3M KAc or NaAc (pH 5.2) and 0.7 volume isopropanol. Mix well and aliquot the sample to 2.0 mL microtubes. 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 10 min. Resuspend the DNA in Elution Buffer or sterile ddH_2O.

The DNA concentration can be determined by a spectrophotometer,

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

Purification of Low-Copy-Number Plasmid andCosmid

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 **400 mL** for the maxiprep.

2. Use **2 x volumes** of the **Buffer A1, Buffer B1** and **Buffer C1** and **100% ethanol**. 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 elute the plasmid DNA. Re-centrifuge or vacuum again if necessary.