Content

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 which enable the high performance of downstream applications such as transfection, restriction mapping, library screening, sequencing, as well as gene therapy and genetic vaccinations.

Important Notes

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 the table below for the 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 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*- strain such as Top 10, DH5a, and C600, we recommend use product number PD1511.

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 with a volume at 4 times 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. The Guaranteed shelf life is 18 months from the date of production.

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: Spin down the RNase A vial briefly. Add the RNase A solution to Buffer A1 and mix well before use. Store at 4 °C.
- Buffer B1 precipitates below room temperature, it is critical to warm up the buffer at 37 °C to dissolve the precipitates before use.
- Buffer N3 may form precipitates below 10 °C, warm up at 37 °C to dissolve the precipitates before use.
- 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.
- Add 96 mL(PD1712-01) or 216 mL(PD1713-03)100 % ethanol into corresponding DNA Wash Buffer。
- ***Carry out all centrifugations at room temperature.***

Materials supplied by users

- 70% ethanol and 100% ethanol.
- High speed centrifuge
- 15 mL high speed centrifuge tubes
- 50 mL tubes

Kit Contents

Catalog #	**PD1712-01**	**PD1712-02**
Preps	10	25
Midi Columns	10	25
Buffer A1	30 mL	70 mL
Buffer B1	30 mL	70 mL
Buffer N3	40 mL	100 mL
EndoClean Buffer	10 mL (Optional: For Endofree)	25 mL (Optional: For Endofree)
Buffer KB	60 mL	130 mL
DNA Wash Buffer	24 mL	54 mL
RNase A(20 mg/mL)	150 µL	350 µL
Elution Buffer	15 mL	30 mL
EndoFree Water	4 mL (Optional: For Endofree)	10 mL (Optional: For Endofree)

EZgene™ HP Plasmid Midiprep Spin Protocol

1. Inoculate ***15- 50 mL*** LB containing appropriate antibiotic with 50 µL fresh starter culture. Grow at 37 °C 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 °C for 6-8 h with vigorous shaking (~250 rpm).
Warning: Do not use more than 50 mL culture or cell mass greater than 150. The buffer volumes need to be scaled up if processing over 50 mL of culture.

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 ***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 5-10 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 ***600 µL Buffer N3***, mix immediately by inverting 5 times and vortex for 10 seconds.

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 12,000 rpm 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 mL Buffer N3*** and ***3 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 ***5 mL*** of the ***lysate/ethonal mixture*** to a DNA column with the collection tube. Centrifuge at 5,000 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/ethonal mixture to the DNA column and centrifuge at 5,000 x g for 1 minute. Discard the flow-through liquid and put the column back to the collection tube. Repeat step "8" till all the ***lysate/ethonal mixture*** has been passed through the column.

9. Add ***5 mL Buffer KB*** into the column, centrifuge at 5,000 x g for 2 minutes at room temperature. Discard the flow-through liquid and put the column back to the collection tube.

10. Add ***5 mL DNA Wash Buffer*** into the column, centrifuge at 5,000 x g for 5 minutes. 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 at 5,000 x g for 10 minutes to remove the ethanol

residues. Transfer the column to the new tube.

Note: It is critical to remove the residual ethanol for optimal yield.

12. Add ***0.3-0.5 mL Sterile ddH$_2$O 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.

    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 if the DNA is used for endotoxin-sensitive cell lines, primary cultured cells or microinjection.

13. Optional: Elute the column with another ***0.5 mL sterile ddH$_2$O or Elution Buffer*** into a new 15 mL tube.

    Note*:* Two elutions give rise to maximum DNA yield. For maximum yield and higher concentration, pool the elutions together, add 0.1 volume 3M potassium acetate or sodium acetate (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,

Purification of Low-Copy-Number Plasmid and 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 volume of the high copy number culture. Use100 mL for the midiprep.

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

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

Endotoxin Removal Procedure（optional）

This protocol is designed to remove endotoxin after the plasmid DNA is purified.

1. Add ***0.1 volume*** of EndoClean Buffer to the plasmid sample in a 15 mL sterile high speed centrifuge tube tube. ***(For example, add 0.1 mL EndoClean Buffer to 1 mL plasmid sample.)***
2. Mix by vortexing the tube a few times and put on ice for about 10 minutes until the solution is clear without turbidity. ***(Rocking the sample in a cold room for 10 minutes is recommended if it is available).***
3. Mix well again by inverting the tube a few times.
4. Incubate the tube at 55℃ water bath for about 5 minutes and the solution shall be turbid. Or add ***0.2 mL*** of ***chloroform*** and mix well by vortexing for 20 seconds.
5. Centrifuge at 12,000 rpm at ***room temperature*** for 5 minutes with the brake off.
6. Carefully transfer the upper clear layer solution to another high speed centrifuge tube.
7. Precipitate plasmid DNA with ***0.1 volume of 3 M NaAc (pH 5.2)*** and ***0.7 volume of Isopropanol.***
8. Centrifuge at 12,000 rpm for 10 minutes. Carefully decant.
9. Add ***1 mL 70% ethanol*** and centrifuge at 12,000 rpm for 5 minutes.
10. Carefully decant and air-dry the DNA for 20 to 30 minutes in a hood.
11. Resuspend the DNA with Endofree ddH_2O or 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: 0.2N 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 Increase the volume of Buffer A1, B1, N3 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.

Limited Use and Warranty

This product is warranted to perform as described in its labeling and in Biomiga's literature when used in accordance with instructions. No other warranties of any kind, express or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by Biomiga. Biomiga's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of Biomiga, to replace the products, Biomiga shall have no liability for any direct, indirect, consequential, or incidental damage arising out of the use, the results of use, or the inability to use it product.

For technical support or learn more product information, please contact us at 400-115-2855 or visit our website at www.biomiga.com.cn