EasyPrep™ EndoFree Plasmid ezFilter Megaprep Protocol

Catalog# PD06-15, PD06-16

Bioland

If crystals form in buffers, warm at 37 °C to dissolve before use

For research use only

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Contents

Introduction..... 3

Important Notes…….. . 3

Storage and Stability..... 4

Before Starting..... 5

Kit Contents 6

Protocol..... 7

Low-Copy-Number Plasmid Purification.... 10

Trouble Shooting Guide 11

Introduction

Key to the kit is our proprietary DNA binding systems that allow the high efficient binding of DNA to our DNA columns while proteins and other contaminates are removed under optimal conditions. Nucleic acids are easily eluted with sterile water or Tris buffer.

Unlike all other rivals, our plasmid purification kit has no guanidine salt in the buffer, the purified DNA is guanidine/ion 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 3 to 5 times. Reference the table below for the commonly used plasmids:

Plasmid	Origin	Copy Numbers	Expected Yield (μg /200 ml)
pSC101	pSC101	5	10-15
pACYC	P15A	10-12	20-25
pSuperCos	pMB1	10-20	20-40
pBR322	pMB1	15-20	30-40
pGEM^{R}	Muted pMB1	300-400	400-500
pBluescript^{R}	ColE1	300-500	400-600
pUC	Muted pMB1	500-700	600-1200

Host strains: The strains used for propagating plasmid have significant influence on yield. Host strains such as Top 10, DH5a, and C600 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 to transform plasmid to an *endA*$^{-}$ strain if the yield is not satisfactory.

***EndA*⁻ Strains of E. Coli**

BJ5182; DH5α; DH1; DH10B; DH20; DH21; JM103; JM105; JM106; JM107; JM108; JM109; MM294; Select96™; SK1590; SK1592; SK2267; SRB; Stbl2™; Stbl4™; XL1-Blue; XL10-Gold; XLO; TOP10

***EndA*⁺ Strains of E. Coli**

ABLE®C; ABLE®K; BL21(DE3); BMH71-18; C600; CJ236; DH12S™; ES1301; HB101; HMS174; JM83; JM101; JM110; KW251; LE392; M1061; P2392; pLysS; PR700; Q358; RR1; TB1; TG1; TKB1; All NM and Y strains

Optimal cell mass (OD_{600} x ml of Culture): The kit is designed for isolating plasmid grown in standard LB medium 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. For over amount of cell numbers, either reduce the biomass or scale up the volumes of Buffer A1, B1 and C1.

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.

Table 2: The optimal cell mass, culture Volume and Binding Capacity for the mega DNA units,

DNA Units	Mega 3	Mega 6	Mega 10
Optimal Cell Mass	1200	2500	4500
Culture Volume	500 ml	1000 ml	1500 ml
Binding Capacity	3-4 mg	6-7 mg	10-12 mg

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 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 6 months when stored at room temperature. Spin down RNase A vial briefly. Add the RNase A solution to Buffer A1 and mix well 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.**
- **Buffer C1 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 by vacuum.

Materials supplied by users:

- 100% ethanol.
- Pump-driven vacuum system, 1,000 ml bottle (Corning# 430518 or 430282) or equivalent pyrex glass bottles.
- 50 ml conical tubes.
- High speed centrifuge tube for endotoxin removal if desired.

Kit Contents

Catalog#	PD06-15	PD06-16
Preps	2	10
DNA Unit	2	10
Filter Unit	2	10
Replacement Cup	4	20
Buffer A1	130 ml	2×320 ml
Buffer B1	130 ml	2×320 ml
Buffer C1	35 ml	160 ml
Buffer RET	130 ml	2×320 ml
DNA Wash Buffer*	50 ml	3×80 ml
RNase A (20 mg/ml)	400 µL	2 ml
Endofree Elution Buffer	50 ml	270 ml
User Manual	1	1

*Add 200 ml (PD06-11) or 320 ml (PD06-12) 96-100% ethanol to each DNA Wash Buffer bottle before use.

Safety Information

- Buffer C1 contains acetic acid, use gloves and protective eyewear when handling.
- Buffer C1, Buffer RET contains chaotropic salts, which may form reactive compounds when combines with bleach. Do not add bleach or acidic solutions directly to the preparation waste.

Plasmid ezFilter Megaprep Protocol

1. **Inoculate 800-1,000 ml LB containing appropriate antibiotic with 500 µl fresh starter culture. Grow at 37°C for 12-16 h with vigorous shaking.** *Prepare a starter culture by inoculating a single colony from a freshly grown selective plate into 1-2 ml LB medium containing the appropriate antibiotic. Grow at 37°C for 6-8 h with vigorous shaking (~300 rpm).*

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

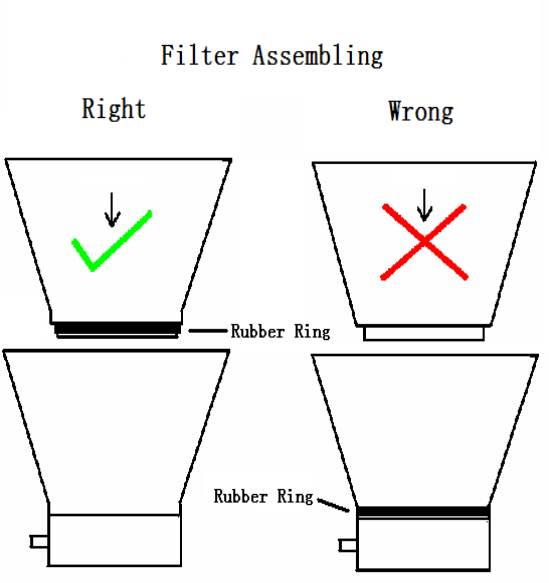
3. **Resuspend the bacterial pellet in 60 ml Buffer A1. Pipet or vortex till the bacterial pellet dispersed thoroughly. Complete resuspension is critical for optimal yields.** *Ensure that RNase A has been added into Buffer A1 before use.*

4. **Add 60 ml Buffer B1. Mix gently but thoroughly by inverting 10 times. Incubate at room temperature for 5 min to obtain a slightly cleared lysate.**

    - *Complete lysis is critical for DNA yield. The mixture of completely lysed bacteria looks transparent.*
    - *Buffer B1 forms precipitation below room temperature, if solution becomes cloudy, warm up at 37°C to dissolve before use.*
    - *Do not incubate longer than 5 min. Over-incubation causes genomic DNA contamination and plasmid damage. Avoid vigorous mixing as this will shear the genomic DNA.*

5. **Add 15 ml Buffer C1 and mix immediately by inverting 5 times till a flocculent white precipitate forms. Vortex the lysate for 5 s. Incubate the mixture at room temperature for 10 minutes.** *It is critical to mix the lysate well, if the mixture still appears conglobated, brownish or viscous; more mix is required to completely neutralize the solution.*

6. **Attach the 2-layer Filter Unit to a sterile 500 ml or 1000 ml standard bottle** (Corning# 430518 or 430282 or equivalent pyrex glass bottle) **and screw tight. Connect the unit to a**



pump-driven vacuum system.

7. **Spin the sample at 5000 rpm for 5 minutes. Transfer the relatively clear lysate of the mixture (use a 50 ml serological pipet) to the filter unit. Stand by for 2 minute and turn on the vacuum.**

   Note 1: Low vacuum force prevents clogging of the filter membranes.

   Note 2: Use a 50 ml serological pipette to transfer the relatively clear lysate from the bottom of the lysate bottle to the filter unit. This will speed up the flow rate of the filter unit. Normally around 120 ml lysate can be filtered through the filter unit within 20-30 min. Pour the remaining white precipitates to the filter unit when most of the lysate has been filtered through.

   Note 3: If the flow-through gets too slow, turn off the vacuum and wait for 1 min. Carefully detach the upper filter cup and replace it with the replacement cup. Assemble the unit as shown in the Figure. Pour the lysate from the original cup to the replacement cup. Turn on the vacuum and filter the rest of the lysate.

8. **When most of the lysate has been filtered through the unit, turn off the vacuum, wait for 1 min, detach the unit and discard the upper filter cup including the rubber rings.** *The DNA is in the solution in the collecting bottle.*

9. **Connect the DNA Binding Unit to a clean 500 ml or 1000ml bottle and screw tight. Connect the Unit to the vacuum system with the vacuum off. Add 60 ml Buffer RET and 50 ml 100% ethanol to the lysate bottle. Mix well by sharp handshaking 3-5 times. IMMEDIATELY pour half of the lysate/ethanol mixture to the DNA binding unit and turn on the vacuum.**

10. **Pour the rest of the lysate/ethanol mixture into the DNA binding unit. When all the lysate pass through the DNA binding unit, vacuum for another 1 min.**

11. **Add 50 ml DNA Wash Buffer evenly to the DNA binding unit and vacuum for 1 min at maximum force. Turn off the vacuum, wait for 1 minute, and discard the liquid waste in the bottle. Reconnect the bottle to DNA binding unit. Repeat once.**

12. **Turn on the vacuum for 30 min at maximum force.** *It is critical to dry the residual ethanol for optimal yield.* **Turn off the vacuum.** *If possible, put the DNA binding cup in 65°C incubator for 15 min to help remove residual ethanol.*

13. **Turn off the vacuum, wait for 1 minute, and replace the 500 ml bottle with a sterile 50 ml conical tube, screw tight.**

14. **Add 15 ml sterile EndoFree Elution Buffer evenly to the membrane and incubate for 2 min. Turn on vacuum to elute DNA. Typically, 8-12 ml of DNA containing solution can be collected. This is the 1st elution.**

15. **Turn off the vacuum and replace the 50 ml conical tube with another sterile 50 ml conical tube, screw tight. Add 10 ml sterile EndoFree Elution Buffer and incubate for 3 min. Turn on the vacuum and collect the 2nd elution.**

Note:

- The DNA is ready for downstream applications such as transfection of endotoxin-sensitive cell lines, primary cultured cells or microinjection.
- 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. Centrifuge at top speed for 10 min. Discard supernatant. Wash the DNA with 1000 µL 70% ethanol, centrifuge for 5 min, carefully decant. Air-dry the pellet for 10-20 minutes in a tissue culture hood.
- Resuspend the DNA in Endofree Elution Buffer. Use less Endofree Elution Buffer if high concentration is desired.

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

Purification of Low-Copy-Number Plasmid

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
2. Use **2 x volume** of the **Buffer A1, Buffer B1 and Buffer C1 and 100% ethanol**. Additional buffers can be purchased from Bioland.
3. use **same volume** of **DNA Wash Buffer** and **Endofree Elution Buffer.**

Trouble Shooting Guide

Problem	Possible Reasons	Suggested improvement
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).
	Bacterial culture overgrown or not fresh	Grow bacterial 12-16 hours. Spin down cultures and store the pellet at -20^{o}C if the culture is not purified the same day. Do not store culture at 4^{o}C over night.
	Low copy-number plasmid	Increase culture volume (up to 3L). 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 aggres-sively after adding buffer B1. Do not incubate more than 5 min after adding solution 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 or DNA doesn't freeze	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.

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