EasyPrep™ Plasmid ezFilter

Megaprep Protocol

Catalog# PD06-09, PD06-10

Bioland

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

For research use only

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Contents

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.

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 purchase.

Before Starting

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

- **RNase A**: 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's critical to warm up at 37-50°C to dissolve the precipitates before use.
- Keep the cap tightly closed for Buffer B1 after use.
- Carry out all centrifugations at **room temperature.**

Materials supplied by users

- 70% ethanol and absolute ethanol (96-100%)
- Vacuum system
- 250 ml, 500 ml bottle (Corning# 430518), 1000 ml bottle (Corning# 430282) or equivalent.
- 50 ml conical tubes

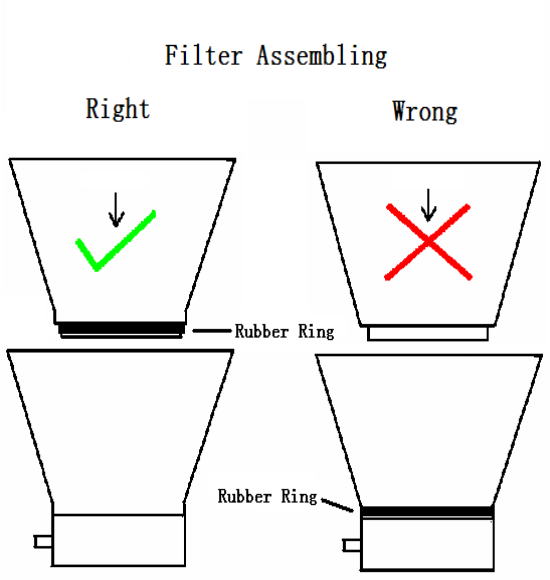
Kit Contents

Catalog Number	PD06-05	D06-06
Preps	2	10
Filter Units	2	10
Filter Unit Replacement Cup	4	20
DNA Binding Units	2	10
Buffer A1	210 ml	2x530 ml
Buffer B1	210 ml	2x530 ml
Buffer C1	250 ml	3x450 ml
Elution Buffer	60 ml	270ml
RNase A	1.1ml	4x1.5 ml
Manual	1	1

- *Buffer C1 contains acetic acid, wear gloves or protective eyewear when handling.*
- *Buffer C1 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 1,200-1,500 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 100 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 100 ml Buffer B1. Mix thoroughly by inverting 10 times with mild shaking. 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. ***Attention:*** 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 120 ml Buffer C1 and mix immediately by inverting 5 times till a flocculent white precipitate forms. Vortex the lysate for 10 s.** 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 and screw tight. Connect the unit to a pump-driven vacuum system (200 mbar).**



7. **Transfer the clear lysate from the bottom of the mixture (use a 50 ml serological pipette) to the filter unit. Stand by for 5 min and turn on the vacuum with low vacuum force.**

   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. Pour the remaining white precipitates to the filter unit when most of the lysate has been filtered through. Normally around 90 ml lysate can be filtered through the filter unit within 10 min.

   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. **Attach the DNA Binding Unit to a clean 500 ml bottle and screw tight. Connect the Unit to the vacuum system with the vacuum off. Add 120 ml 96-100% ethanol to the lysate bottle and mix well. 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 2 min.**

11. **Add 50 ml 70% ethanol evenly to the DNA binding unit and vacuum for 1 min. Turn off the vacuum, wait for 1 min, and discard the liquid waste in the bottle. Reconnect the bottle to DNA binding unit. Repeat .**

12. **Add 80 mL 100% ethanol evenly to the DNA membrane and vacuum for 1minute. Turn on the vacuum for 20 min at maximum force (It is critical to dry the residual ethanol for optimal yield). Turn off the vacuum and wait for 1 min. Replace the 500 ml bottle with a sterile 50 ml conical tube, screw tight.**

13. **Add 12 ml sterile ddH_2O or Elution Buffer evenly to the membrane and incubate for 2 min. Turn on vacuum to elute DNA. Typically, 3-5 mL of DNA containing solution can be collected. This is the 1st elution.**

14. **Turn off the vacuum and replace the 50 mL conical tube**

with another sterile 50 mL conical tube, screw tight. Add 8 mL sterile ddH2O or Elution Buffer and incubate for 1 minute. Turn on the vacuum and collect the 2nd elution, typically 5-6 mL of solution can be collected.

Note 1: If ddH2O is used for eluting DNA, make sure the pH is ≥ 7.0.

Note 2: The DNA is ready for down stream applications such as cloning or transfection of HEK293 cells.

Note 3: It's highly recommended to remove the endotoxin if the DNA is used for endotoxin-sensitive cell lines, primary cultured cells or microinjection.

Note 4: For maximum yield and higher concentration, combine the 2 elutions, precipitate the DNA with 0.1 volume of 3M KAc, pH 5.2 and 0.7 volume of isopropanol. Centrifuge the sample at top speed for 10 min, carefully decant. Wash the DNA with 70% ethanol, centrifuge at top speed for 5 min, carefully decant. Air-dry the sample and resuspend the sample in sterile water or TE buffer accordingly.

DNA concentration = Absorbance 260 nm x 50 x dilution factor (µg/ml)

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°C if the culture is not purified the same day. Do not store culture at 4°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 aggressively 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.

Table: Other related products

Catalog #	Product Name	Preps
PD01-01	Plasmid Miniprep kit	50
PD01-02	Plasmid Miniprep kit	250
PD02-01	Plasmid Miniprep-II kit	50
PD02-02	Plasmid Miniprep-II kit	250
PD03-01	Plasmid Midiprep kit	10
PD03-02	Plasmid Midiprep kit	25
PD04-01	Plasmid Midiprep-II kit	10
PD04-02	Plasmid Midiprep-II kit	25
PD03-11	Plasmid ezFilter Midiprep kit	10
PD03-12	Plasmid ezFilter Midiprep kit	25
PD04-11	Plasmid ezFilter Midiprep-II kit	10
PD04-12	Plasmid ezFilter Midiprep-II kit	25
PD05-01	Plasmid Maxiprep kit	10
PD05-02	Plasmid Maxiprep kit	25
PD05-11	Plasmid ezFilter Maxiprep kit	10
PD05-12	Plasmid ezFilter Maxiprep kit	25
PD03-21	EndoFree Plasmid Midiprep kit	10
PD03-22	EndoFree Plasmid Midiprep kit	25
PD03-31	EndoFree Plasmid ezFilter Midiprep kit	10
PD03-32	EndoFree Plasmid ezFilter Midiprep kit	25
PD04-21	EndoFree Plasmid Midiprep-II kit	10
PD04-22	EndoFree Plasmid Midiprep-II kit	25
PD04-31	EndoFree Plasmid ezFilter Midiprep-II kit	10
PD04-32	EndoFree Plasmid ezFilter Midiprep-II kit	25
PD05-21	EndoFree Plasmid Maxiprep kit	10
PD05-22	EndoFree Plasmid Maxiprep kit	25
PD05-31	EndoFree Plasmid ezFilter Maxiprep kit	10
PD05-32	EndoFree Plasmid ezFilter Maxiprep kit	25

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