Contents

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 contaminates are removed under certain optimal conditions. Nucleic acids are easily eluted with sterile water or elution buffer.

Unlike other procedures, our patented 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

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 500 mL)
pSC101	pSC101	5	50-60
pACYC	P15A	10-12	80-100
pSuperCos	pMB1	10-20	80-150
pBR322	pMB1	15-20	100-150
pGEM®	Muted pMB1	300-400	2000-2500
pBluescript®	ColE1	300-500	2000-3000
pUC	Muted pMB1	500-700	3000-4000

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 PD1714.

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.

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 3 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 and Buffer ER should be stored at 4℃. All other materials can be stored at room temperature (22-25℃). The Guaranteed shelf life is 12 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 steps.

Important:

- **RNase A**: It is stable for more than half a year 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.**
- The proper volume of buffer ratio of A1:B1:C1: 100% ethanol =1:1:1.2:1.2.
- 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, 500 mL bottle or 1,000 mL bottle (Corning# 430518 or 430282) or equivalent pyrex glass bottles.
- 50 mL conical tubes.

Kit Contents

Catalog#	PD1612-00	PD1612-01	PD1612-02
Preps	1	2	10
DNA Unit	1	2	10
Filter Unit	1	2	10
Replacement Cup	1	4	20
Buffer A1	65 mL	130 mL	2 x 320 mL
Buffer B1	65 mL	130 mL	2 x 320 mL
Buffer C1	75 mL	160 mL	2 x 400 mL
DNA Wash Buffer*	24 mL	54 mL	3×80 mL
RNase A (20 mg/mL)	6.5 mg (325 µL)	13 mg (650 µL)	64 mg (2x1.6mL)
Elution Buffer	30 mL	60 mL	270 mL
User Manual	1	1	1

*Add 96 mL (PD1612-00) or 216 mL (PD1612-01) or 320 mL (PD1612-02) 96-100% ethanol to each DNA Wash Buffer bottle before use.

Safety Information

- Buffer C1 contains acidic acid, wear gloves and 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.

EZgene™ Plasmid ezFilter Megaprep 6 Protocol

1. Inoculate **800-1,200 mL** LB containing appropriate antibiotic with 500 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). The buffer volumes need to be scaled up if processing over 1,000 mL of culture.
   Note: Do not use a starter culture that has been stored at 4°C.
   Note: Do not grow starter culture directly from glycerol stock.

2. Harvest **800-1,200 mL** overnight bacterial cells by centrifugation at 5,000 x g for 10 minutes at room temperature. Decant or aspirate medium and discard.

   Note: Remove the residual medium completely for optimal cell lysis and neutralization.

3. Resuspend the bacterial pellet in **60 mL Buffer A1** (Add **RNase A** to **Buffer A1** before use). Pipet or vortex till the bacterial pellet dispersed thoroughly **(Complete resuspension is critical for optimal yields)**.

4. Add **60 mL Buffer B1,** mix gently but thoroughly by inverting 10 times and incubate at room temperature for 5 minutes to obtain a cleared lysate.

   Note: Do not incubate longer than 5 minutes. Over-incubating causes genomic DNA contamination and plasmid damage. Avoid vigorous mixing as this will shear the genomic DNA.

5. Add **72 mL Buffer C1** and mix immediately by inverting 5 times and sharp hand shaking for 10 times till a flocculent white precipitate forms. Incubate the mixture at room temperature for 10 minutes.

   Note: 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. Transfer the clear lysate from the bottom of the mixture (use a 50 mL serological pipet) to the filter unit. Stand by for 5 minutes and turn on the

vacuum with low vacuum force and increase to maximum vacuum force after 5 minutes.

Note 1: Low vacuum force prevents clogging of the filter membranes.
Note 2: Use a 50 mL serological pipet 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 80 mL lysate can be filtered through the filter unit within 10-15 minutes. Pour the remaining white precipitates to the filter unit when most of the lysate has been filtered through.

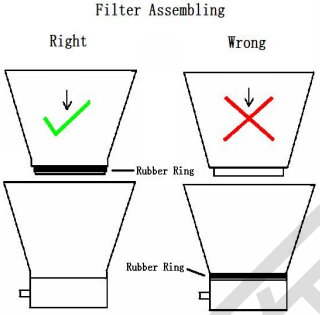


Figure 1. Instruction of filter assembling.

Note 3: If the flow through gets too slow, turn off the vacuum and wait for 1 minute. Carefully detach the upper filter cup and replace it with the replacement cup. Assemble the unit as **Figure 1**. 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 minute, detach the unit and discard the upper filter cup including the rubber rings.

   Note: The DNA is in the collection bottle.

9. Connect the DNA unit to a 500 mL or 1000 mL standard bottle and screw tight. Connect the DNA unit to the vacuum with the vacuum off. Add **72 mL 100% ethanol** to the lysate bottle. Mix well by sharp hand shaking 3-5 times and immediately pour **half** of the **lysate/ethanol mixture** to the DNA unit and turn on the vacuum.

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

11. Wash the DNA membrane with **50 mL DNA Washing Buffer** and vacuum for 1 minute at maximum force. Repeat this step once.

12. Add **60 mL 100% ethanol** evenly to the DNA membrane and vacuum for 1 minute. Turn off the vacuum, wait for 1 minute, and discard the liquid waste in

the bottle. Reconnect the bottle to the DNA binding unit. Turn on the vacuum for 10 minutes at maximum force to remove the ethanol residues. Turn off the vacuum, incubate at 65ºC for 15 min will help to remove the ethonal and increase the elution efficiency.

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

13. Replace the 500 mL or 1000 mL bottle with a sterile 50 mL conical tube, screw tight.

14. Add **10 mL sterile ddH$_2$O or Elution Buffer** evenly to the membrane and incubate for 2 minutes. Turn on vacuum to elute DNA. Typically, **3-5 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 **8 mL sterile ddH$_2$O or Elution Buffer** and incubate for 1 minute. Turn on the vacuum and collect the 2nd elution, typically **3-5 mL** of solution can be collected.

Note: If ddH$_2$O 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 robust cell lines (HEK293 cells).
Note: It's highly recommended to remove the endotoxin (PD1615) if the DNA is used for 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. 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 Elution Buffer or sterile ddH$_2$O.

DNA concentration (µg/m L) = $OD_{260\ nm}$ x 50 x dilution factor.

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 volumes** of the high copy number culture.
2. Use **2 x volume** of the **Buffer A1, Buffer ER,Buffer B1,Buffer C1 and 100% ethanol**. Additional buffers can be purchased from Biomiga.
3. Use **same volume** of **DNAWash Buffer** and **Elution Buffer.**

质粒超大量提取试剂盒 6 简明步骤（PD1612）

（详细内容请参考说明书英文部分）

I. 实验前准备

RNase A：室温下可稳定贮藏半年，长期贮藏请置于4ºC保存。使用前将提供的所有RNase A瞬时离心后加入Buffer A1, 使用后将Buffer A1/RNase A置于4ºC保存。

Buffer B1：在低于室温时会沉淀，请于50ºC左右水浴加热至沉淀完全溶解，溶液澄清，使用后保证Buffer B1瓶盖旋紧。

Buffer C1：低于10ºC会沉淀，请于37 ºC左右水浴加热至沉淀完全溶解，溶液澄清。

准备100%乙醇

准备100%的乙醇，在室温下（22-25ºC）进行所有离心操作。

II. 注意事项

质粒拷贝数：纯化中低拷贝的质粒时，使用2倍的菌液体积，2倍的Buffer A1,B1,,C1,100%乙醇，相同体积的DNA Wash Buffer和Endofree Elution Buffer 转化菌：若为-70ºC甘油冻存的菌，请先涂布平板培养后，再重新挑选新的单个菌落进行培养。切勿直接取冻存在4ºC的菌进行培养。

柱结合能力：6 ~7mg

III. 操作步骤

1. 取500 µL新鲜的菌液接种到 **800-1200 mL** (勿超过 1,000 mL)的LB培养基（含适量抗生素），37ºC震荡培养14-16小时。室温下5,000 x g离心10分钟，收集菌体，并尽可能的吸去上清。

   注： 残留的液体培养基容易导致菌液裂解不充分。

   注： 本说明书中的操作程序适用于标准 LB （Luria Bertani）培养基培养 12-16 小时后，OD_{600}（细菌密度）在 2.0-3.0 之间的菌液。若采用的是富集培养基，例如 TB 或 2×YT，请注意保证 OD_{600} 不超过 3.0。

2. 加入**60 mL Buffer A1**（确保已加入RNase A），用移液器或涡流震荡确保细菌沉淀重新悬浮。

   注： 不完全悬浮易导致菌体裂解不完全，从而使产量降低。

3. 加入 **60 mL Buffer B1**, 轻轻地反转5-10 次以混合均匀，然后静置2-5分钟至溶液粘稠而澄清。

   注： 切勿剧烈振荡。静置时间不应超过5分钟，时间过长会导致基因组DNA污染或质粒受到破坏。若溶液未清亮澄清，则表明菌体裂解不充分，应加大Buffer B1

的用量或减少菌体量。

4. 加入 **72 mL Buffer C1,**立即反转几次至出现絮状白色沉淀。涡漩震荡10秒。充分混匀后，溶液将会变得颜色均一，如果此时溶液颜色不均一，均局部颜色过深，建议将溶液轻甩几次，以充分混合溶液，室温下静置10分钟。

注意：将溶液混合均匀非常关键，如出现球状体或黏稠状，继续震荡中和溶液。

5. 将双层 **Filter unit** 与 500 mL或 1000 mL的**灭菌瓶 (Corning# 430518 or 430282 or equivalent)** 连接，旋紧，再将此装置与真空负压装置连接。

6. 先将步骤“5”中底部较澄清的裂解产物用50 mL移液管转移至双层**Filter unit**中，静置5分钟后打开真空装置，使负压由低到高，5分钟后增至最大（0.04 Mpa）。

注1：开始用较低压力可避免过滤膜堵塞。

注2：先用50 mL的管转移底部相对较为澄清的裂解液至双层*Filter unit*中，通常情况下，10-15分钟可过滤80 mL的裂解液。大部分裂解液过滤完后，最后将白色沉淀也全部倒入真空过滤装置中。

注3： 如果如果过滤速度变的很慢，关掉真空，等待1分种后取下上部的过滤杯，换一个新的过滤杯，操作方法如上图所示，并将原来杯中的溶液转移至新的过滤杯中，开启负压装置，过滤完剩余的溶液。

7. 当大部分裂解液已被过滤时，关掉真空负压装置，等候1分种，拆卸装 置，移去双层**Filter unit**。

注意：此时质粒DNA在收集瓶中。

8. 再将**DNA unit**杯与一个新的灭菌的500ml或1000ml螺口标准瓶连接，旋紧。并将过滤嘴与真空负压装置连接。将步骤“7”中收集到的裂解液中加入**72 mL 100%乙醇**，立即混合均匀，**立即**将一半倒入**DNA unit**杯中，开启负压装置，进行过滤吸附操作。

注意：此裂解液与乙醇的混合液，应该立刻进行过滤吸附操作，长时间放置将会降低吸附率，严禁长时间放置。

9. 再将剩下一半倒入**DNA unit杯**中，当所有裂解液已过滤完，再维持真空1分钟后，关掉负压装置。

10. 在**DNA Unit杯**中加入**50 mL DNA Washing Buffer**，开启负压装置至最大（0.04Mpa）并维持1分钟，使乙醇完全通过DNA Unit杯。**重复此操作步骤一次**。

11. 在**DNA Unit杯**中加入**60 mL 100%乙醇**，开启最大负压维持1分钟，关掉负压装置，倒掉瓶子中的废液，将DNA Unit杯重新连接至标准瓶上，开启负压装置至最大负压，真空抽10分钟。关掉负压装置，移去标准瓶，将DNA Unit杯 置于65度烘箱中放置15min，以充分去除残留的乙醇。

12. 将**DNA Unit** 连接至一个新的**50 mL**的离心管中，并旋紧。

13. 加入**10 mL 灭菌 ddH2O或者Elution Buffer**到**DNA Unit杯**的膜上，室温

放置2分钟，打开负压装置至最大（0.04 Mpa）洗脱DNA。此时会收集到**3~5 m L**洗脱液，这是**1st**洗脱液。

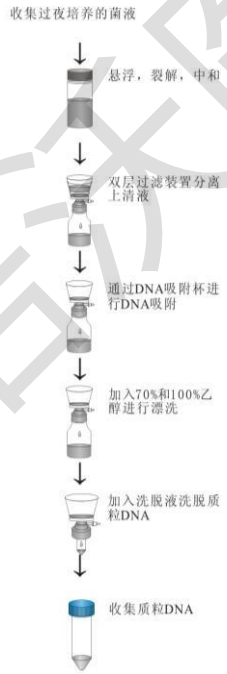
14. 关掉负压装置，将DNA Unit连接至一个新的50mL的离心管中，加入**8 mL 灭菌 ddH2O或者Elution Buffer**到**DNA Unit杯**的膜上，室温静置2分钟，开启负压装置至最大（0.04 Mpa）洗脱DNA。此时会收集到约**3-5 mL**洗脱液，这是**2nd**洗脱液。

注1：提取到的质粒DNA可直接用于基因克隆、测序、酶切、文库筛选、体外转录翻译、转染HEK293细胞。

注2：如果提取到的质粒用于转染内毒素敏感性细胞株，原代细胞及用于微注射我们建议去除内毒素。

注3：此两步洗脱可以基本收集到全部的质粒DNA，如果想得到高收获量和高浓度，建议进行以上的两步洗脱，然后参照以下进行浓缩操作。合并以上洗脱液，加0.1倍体积的3M的醋酸钠（pH5.2）和0.7倍体积的异丙醇，室温下高速离心10分钟，弃去上清，加入1 mL 70% 乙醇，高速离心5分钟，弃去上清，空气干燥10~20分钟，加入去离子水或Elution Buffer重新溶解质粒DNA。

IV. 操作流程图



Trouble Shooting Guide

Problems	Possible Reasons	Suggested Improvements
Low Yield	Poor Cell lysis.	• Resuspend pellet thoroughly by votexing and pipeting 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 4oC over night.
Low Yield	Low copy-number plasmid.	Increase culture volume to 2 x of original 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 solution 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.