

EZgene™ Endofree Plasmid Midiprep Kit II
(BW-PD1418)

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Kit Contents

Catalog#	BW-PD1418-00	BW-PD141801	BW-PD1418-02
Preps	2	10	25
EzBind™ Columns	2	10	25
Filter Syringe (20 mL)	2	10	25
Buffer GBL	7.5 mL	30 mL	70 mL
Buffer A1	12 mL	60 mL	140 mL
Buffer B1	12 mL	60 mL	140 mL
Buffer N3	16 mL	80 mL	200 mL
EndoClean Buffer	4 mL	20 mL	50 mL
DNA Wash Buffer*	5 mL	24 mL	54 mL
Endofree Elution Buffer	6mL	30 mL	100 mL
RNase A (20 mg/mL)	60 µL	300 µL	700 µL
User Manual	1	1	1

*Add 25 mL (PD1418-00) or 96 mL (PD1418-01) or 216 mL (PD1418-02) 96-100% ethanol to each DNA Wash Buffer bottle before use.

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.

Plasmid isolated with traditional protocol normally contains high level of endotoxins (Lipopolysaccharides or LPS). For transfection of endotoxin sensitive cell lines or microinjection, the endotoxins should be removed before the applications. The EZgene™ endofree system uses a specially formulated buffer that extracts the endotoxin from the plasmid DNA. Two rounds of extraction will reduce the endotoxin level to 0.1 EU (Endotoxin) per µg of plasmid DNA. The endofree plasmid miniprep kit provides an efficient endotoxin removal step into the traditional

purification procedure to produce transfection grade plasmid DNA.

This kit is designed for fast and efficient purification of plasmid DNA from 50 to 100 mL of *E. coli* culture. The midi column has a DNA binding capacity of 400 µg. The purified endofree DNA is ready for downstream applications such as transfection of endotoxin-sensitive cell lines, primary cultured cells or microinjection.

Important Information

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 accordingly. Please contact our customer service for further information and refer to Table 1 for the commonly used plasmids.

Table 1 Commonly used plasmids and expected yield.

Plasmid	Origin	Copy Numbers	Expected Yield (µg per 100 mL)
<i>pSC101</i>	<i>pSC101</i>	5	5
<i>pACYC</i>	<i>p15A</i>	10-12	10-12
<i>pSuperCos</i>	<i>pMB1</i>	10-20	10-20
<i>pBR322</i>	<i>pMB1</i>	15-20	15-20
<i>pGEM^R</i>	<i>Muted pMB1</i>	300-400	300-400
<i>pBluescript^R</i>	<i>ColEI</i>	300-500	300-500
<i>pUC</i>	<i>Muted pMB1</i>	500-700	500-700

Host Strains: The strains used for propagating plasmid have significant influence on yield. The strains used for propagating plasmid have significant influence on yield. Host strains such as *TOP10*, *DH5α* 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 recommended transform plasmid to an *endA⁻* strain if the yield is not satisfactory. Please refer to Table 2 for the *endA* information.

Table 2 *endA* strains of *E. coli*.

<i>End A⁻</i> Strains of <i>E. coli</i>							
<i>DH5α</i>	<i>DH1</i>	<i>DH21</i>	<i>JM106</i>	<i>JM109</i>	<i>SK2267</i>	<i>SRB</i>	<i>XLO</i>
<i>TOP10</i>	<i>DH10B</i>	<i>JM103</i>	<i>JM107</i>	<i>SK1590</i>	<i>MM294</i>	<i>Stbl2TM</i>	<i>XL1-Blue</i>

<i>BJ5182</i>	<i>DH20</i>	<i>JM105</i>	<i>JM108</i>	<i>SK1592</i>	<i>Select96™</i>	<i>Stbl4™</i>	<i>XL10-Gold</i>
End A⁺ Strains of <i>E. coli</i>							
<i>C600</i>	<i>JM110</i>	<i>RR1</i>	<i>ABLE®C</i>	<i>CJ236</i>	<i>KW251</i>	<i>P2392</i>	<i>BL21(DE3)</i>
<i>HB101</i>	<i>TG1</i>	<i>TB1</i>	<i>ABLE®K</i>	<i>DH12S™</i>	<i>LE392</i>	<i>PR700</i>	<i>BL21(DE3)</i> <i>pLysS</i>
<i>JM101</i>	<i>JM83</i>	<i>TKB1</i>	<i>HMS174</i>	<i>ES1301</i>	<i>M1061</i>	<i>Q358</i>	<i>BMH71-18</i>
All NM Strains				All Y Strains			

Optimal Cell Mass (OD₆₀₀ × 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 OD₆₀₀ 2.0 to 3.0. If rich medium such as TB or 2 × YT are used, make sure the cell density doesn't exceed 3.0 (OD₆₀₀). A high ratio of biomass over lysis buffers result in low DNA yield and purity. The Midi Column II has an optimal biomass of 150-250. For example, if the OD₆₀₀ is 2.5, the optimal culture volume should be 50 to 100 mL. For over amount of cell numbers, either reduce the biomass or scale up the volume of Buffer A1, B1 and C1.

Culture Volume: Use a flask or tube 4 times bigger 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 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 (15-25°C). 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 user manual and become familiar with each step and pay special attention to the followings.

Important Notes

- ☼ RNase A: 20 mg/mL. It is stable for one year at room temperature (15-25°C). Spin down RNase A vial briefly before adding to Buffer A1.
- ☼ Buffer A1 should be stored at 4°C once RNase A is added.
- ☼ Buffer B1 precipitates below room temperature. It is critical to warm up the buffer 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, especially, after mixing the lysate with ethanol, the sample needs to be processed immediately by centrifugation.
- ☼ Centrifuge speed at 5,000 x g is recommended. In case the collection tube doesn't fit in high speed centrifuge rotor, use benchtop centrifuge and spin at 2,500 x g with double centrifugation time.
- ☼ *Carry out all centrifugations at room temperature.*

Materials not Supplied

- ☼ High speed centrifuge.
- ☼ 96-100% ethanol.
- ☼ 15mL and 50 mL centrifugal tubes.

Safety Information

Buffer N3 contains acidic acid, wear gloves and protective eyewear when handling. Do not add bleach or acidic solutions directly to the preparation waste.

EZgene™ EndoFree Plasmid Midiprep Spin Protocol

A. Removal of Endotoxin *during* Plasmid Purification

This protocol is designed for removing the endotoxin during the plasmid purification.

1. Inoculate **50-100 mL** LB containing appropriate antibiotic with 50 µL fresh starter culture.

Incubate at 37 °C for 14-16 hours 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 hours with vigorous shaking (~250 rpm).

Note: Do not use a starter culture that has been stored at 4°C.

Note: Do not grow starter culture directly from glycerol stock.

Note: Do not use more than 50 mL culture or cell mass greater than 150.

2. Column equilibration: Place a **EzBind™ Column** in a clean collection tube, and add **2.5mL Buffer GBL** to column. Centrifuge for 1 min at 8000 rpm in a table-top centrifuge. Discard the flow-through, and set the column back into the collection tube. (Please use freshly treated spin column).

3. Harvest the bacterial culture by centrifugation for 10 minutes at 5,000 ×g. Pour off the supernatant and blot the inverted tube on a paper towel to remove residue medium. Remove the residue medium completely.

Note: Residue medium will cause poor cell lysis and thus lower DNA yield.

4. Add **5 mL Buffer A1** (*Add RNase A to Buffer A1 before use*) and completely resuspend bacterial pellet by vortexing or pipetting.

Note: Complete resuspension is critical for bacterial lysis and lysate neutralization.

5. Add **5 mL Buffer B1**, mix gently by inverting the tube 10 times (*do not vortex*), and incubate at room temperature for 5 minutes.

Note: Do not incubate for more than 5 minutes.

Note: Buffer B1 precipitates (cloudy look) below room temperature. Warm up Buffer B1 at 37°C to dissolve the precipitations before use.

6. Add **1.2 mL Buffer N3**, mix completely by inverting/shaking the vial for 5-10 times.

Note: Incubating the lysate in ice for 1 minute will improve the yield.

Note: It is critical to mix the solution well. If the mixture still appears conglobated, brownish or viscous, more mixing is required to completely neutralize the solution.

7. Two options for clearing the lysates:

High Speed centrifuge: Transfer the lysate to a high speed centrifuge tube and centrifuge at 8000 rpm for 10 minutes at room temperature. Transfer the cleared lysate to a 15 mL centrifugal 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.

ezFilter Syringe: Pour the lysate directly into the barrel of the filter syringe. Insert the syringe to a clean 15 mL centrifugal 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 centrifugal 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.**

8. Transfer the clear lysate to a new high-speed centrifuge tube and add **0.1 volume** of EndoClean Buffer, vortex for 10s and incubate on ice for 10 minutes. Mix the sample several times without leaving ice.

Note: Use a serological pipet or a tip cut with a clean razor in the end to transfer the EndoClean Buffer.

Note: At room temperature (> 23°C), the sample becomes turbid after adding EndoClean Buffer. The solution becomes clear after incubating on ice.

9. Centrifuge at 8000 rpm for 10 min at room temperature (the temperature must be greater than 23 °C). The solution should separate into 2 phases, the upper clear phase contains DNA and the lower organic phase contains endotoxin. The two phases will not separate if the temperature is less than 23 °C.

Note: If phase partitioning is not observed after centrifugation:

Incubate the solution at 65°C for 5 minutes. The solution becomes turbid again. And then repeat step 9 or add 200 µL Chloroform (37°C), vortex to mix well, repeat step 9.

Note: Up to 99% of the endotoxin can be removed by extracting with the EndoClean buffer once. Another extraction is necessary if less than 0.1 EU (Endotoxin)/ µg of DNA is desired by repeating step 8-9.

10. Carefully transfer the clear lysate into a 15 mL centrifugal tube, avoid the precipitations. Add **6 mL Buffer N3** and **6 mL 100% ethanol**. Mix immediately by sharp shaking. The mixture of ethanol/lysate needs to be centrifuged through the **EzBind™ Column** immediately.
11. Immediately apply **6 mL** of the lysate/ethanol mixture to a pretreatment **EzBind™ Column**

with the collection tube. Centrifuge at 8000 rpm 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 column and centrifuge at 8000 rpm for 1 minute. Discard the flow-through liquid and put the column back to the collection tube.

12. Add **5 mL DNA Wash Buffer** (*Add ethanol to DNA Wash Buffer before use*) into the **EzBind™ Column**, centrifuge at 8000 rpm for 1 minute, discard the flow-through.

13. Add **5 mL** 100% ethanol into the **EzBind™ Column**, centrifuge at 8000 rpm for 1 minute, discard the flow-through.

14. Reinsert the **EzBind™ Column**, with the lid open, into the 15 mL centrifugal tube and centrifuge for 10 minutes at 8000 rpm.

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

15. Carefully transfer the spin **EzBind™ Column** into a clean 15 mL tube and add **0.5-1.0 mL Endofree Elution Buffer** to the center of the column and incubate for 1 minute at room temperature. Elute the DNA by centrifugation at 8000 rpm for 5 minutes. If higher yield is desired, reload the eluate in the 15 mL tube to the column, incubate for 1 minute and centrifuge again.

Optional: Reload the eluate into the center of the column for a second elution.

Note: Two elutions give rise to maximum DNA yield. Use less Endofree Elution Buffer if high concentration is desired.

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

16. The DNA concentration can be calculated as follows,

Concentration (µg/mL)=OD₂₆₀×50×dilution factor.

B. Removal of Endotoxin *after* Plasmid Purification

This protocol is designed for removing the endotoxin after the plasmid is purified.

1. Follow the protocol from Step 1 to 7 on page 6-7.
2. Transfer the lysate to a clean 15 mL conical tube and add **6 mL** of **Buffer N3** and **6 mL** of **100% ethanol**, mix well and go to step 11-15 on page 8.
3. After the plasmid is purified, add **0.1 volume** of **EndoClean Buffer** to the plasmid sample in a 2 mL centrifuge tube (For example, add **0.1 mL EndoClean Buffer** to **1 mL plasmid**

sample). The solution becomes turbid after adding EndoClean Buffer.

4. Vortex the tube for 5s and put on ice for about 10 minutes Mix the sample several times without leaving ice. The solution becomes clean after incubating on ice.
5. Centrifuge at 8000 rpm at room temperature for 10 minutes **(the temperature must be greater than 23°C for phase partitioning)**.

Note: If phase partitioning is not observed after centrifugation.

- Incubate the solution at 65°C for 5 minutes. , and repeat step 5.
- Or add **200 µL Chloroform** (37°C), vortex for 10s, and repeat step 5.

6. Carefully transfer the upper clear layer solution to a 2 mL tube.
7. Precipitate plasmid DNA with **0.1 volume** of **3 M KAc (pH 5.2)** and **0.7 volume** of **Isopropanol**. Centrifuge at 8000 rpm for 10 minutes. Carefully decant.
8. Add **2 mL 70% ethanol** and centrifuge at 8000 rpm for 5 minutes. Carefully decant and air-dry the DNA for 30 minutes in a hood.
9. Resuspend the DNA with **Endofree Elution Buffer**.

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

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

Purification of Low-Copy-Number Plasmid/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:

- ☼ Culture volume: Use **2×volumes** of the high copy number culture. Use **100 mL** for the midiprep.
- ☼ Use **2×volumes** of the **Buffer A1**, **Buffer B1** and **Buffer N3**. Additional buffers can be purchased from Biomiga.
- ☼ Use **same volume** of **DNA Wash Buffer** and **Elution Buffer**.

无内毒素质粒中提过滤法 II 型简明步骤 (PD1418)

(详细内容请参考说明书英文部分)

I. 实验前准备

RNase A: 室温下可稳定保存半年, 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4°C保存。

Buffer B1: 在低于室温时会沉淀, 请于50°C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 N3: 低于10°C会沉淀, 请于37 °C左右水浴加热至沉淀完全溶解, 溶液澄清。

准备100%的乙醇。

在室温下 (22-25°C) 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,N3,100%乙醇, 相同体积的DNA Washing Buffer 和Endofree Elution Buffer..

转化菌: 若为-70°C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4°C的菌进行培养。

柱结合能力: 500ug

对富含内源核酸酶的宿主菌 (endA+) 如HB101, JM101, TG1等, 需去核酸酶, 请使用产品PD1712。

III. 操作步骤 (在提取质粒过程中去除内毒素)

1. 取50 µL新鲜的菌液接种到**50-100 mL** (勿超过 50 mL)的LB培养基 (含适量抗生素), 37°C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, 离心后沉淀较松, 不能有效吸取上清。

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, OD600 (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 OD600 不超过 3.0。

2. 柱平衡: 向吸附柱EzBind™ Column 中加入2.5mL平衡液 Buffer GBL, 8000 rpm 离心1分钟, 弃去收集管中的滤液, 将吸附柱重新放回收集管中备用。(处理完请于当天使用)
3. 加入**5 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。

4. 加入 **5 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。

5. 加入 **1.2 mL Buffer N3**，立即反转5次，用手用力摇晃3-5次充分混匀，此时出现白色絮状沉淀。

6. **方法一：**将离心管转至高速离心机，在**室温下**8000 rpm 离心10分钟（若上清中

有白色沉淀，可再次离心）小心吸取离心后的上清液至 15 mL 管中（避免吸起沉淀）。

注：低温下 RNase 不工作，易有 RNA 污染。如果离心机转子较冷，将离心管**室温下**温育 10 分钟后再离心。

7. **方法二：**将裂解液转移至过滤器中，放在一个15 mL的试管上静置10分钟。管中的白色絮状沉淀浮上来，对准15 mL的管向下压，使裂解液尽可能多的通过，有些裂解液可能会残留在沉淀中。

注：静置10 分钟时RNase A将工作，排除RNA污染。

8. 定量吸取离心后的上清液至新的15 mL管中（避免吸起沉淀），加入**0.1 倍体积的EndoClean Buffer**，混匀后冰浴10分钟，其间不时摇匀(若**EndoClean Buffer**粘稠难吸，可将枪头剪掉头后再吸取)。

注：加入**EndoClean Buffer**后溶液变红并混浊，冰浴后变清亮。

9. 室温下(冰浴取出后务必使溶液温度恢复到23℃以上，否则溶液不分层)8000 rpm 离心10分钟（也可在2,500 x g离心15 min)。此时溶液分为两层，上层水相含有质粒，下层红色有机相含有内毒素。

注：若分层不明显，则是因为温度不够，可65℃温浴5分钟，再在8000 rpm 离心10分钟。或者加入经温育后的200 μL Chloroform (>37℃)，混匀后再重复步骤9。

注：此时可去除99%的无内毒，再重复第8-9步可使内毒素含量低于0.1 EU(Endotoxin)/ μg DNA.

10. 将上层水相转移至一个新的15 mL管中，加入**6 mL 的Buffer N3**及**6 mL 的100% ethanol**，用手用力甩5次以混匀，该混合溶液需要马上进行过吸附柱操作。

11. 立即转移**6.0 mL**裂解液至带收集管的吸附柱中，室温下8000 rpm 离心1分钟，倒掉收集管中的废液，将吸附柱重新放回到收集管中。重复此步直至所有的溶液通过吸附柱。

12. 向吸附柱中加入**5 mL DNA Washing Buffer**，室温下8000 rpm 离心1分钟，倒掉收集管中的废液，将吸附柱重新放回到收集管中。重复步骤“10”。

13. 向吸附柱中加入**3 mL 100% 乙醇**，室温下8000 rpm 离心1分钟，倒掉收集管

中的废液，将吸附柱重新放回到收集管中。

14. 将吸附柱放回高速离心机中，室温下8000 rpm **开盖**离心10分钟。离心后，将吸附柱在 65 度烘箱中放置 10 分钟有助于彻底去除乙醇，提高 **Endofree Elution Buffer** 的洗脱效率。

IV. 操作步骤（在提取质粒后去除内毒素）

该步骤用于质粒纯化后内毒素去除。

1. III 操作步骤中 1-7 步进行（第 10-11 页），转移上清至新的 15 mL 离心管中。加入 **6 mL Buffer N3** 和 6 mL 100%乙醇。混匀。之后按照 III 操作步骤中的 11-14 步继续实验操作，得到质粒。
2. 加入 0.1 倍体积的 **EndoClean Buffer** 至含质粒样品的 2.0 mL 无菌高速离心管（例如，加入 0.1 mL EndoClean Buffer 至 1 mL 质粒样品）。
3. 涡旋几次，冰上静置 10 min，直至溶液澄清不浑浊。（若可行的话，建议将样品置于冷室震荡 10 min）颠倒混匀。
4. 室温 10,000 ×g 离心 10 min。若离心温度低于 23℃，将不会见到分层现象。
注：若离心后没有见到分层现象，65℃孵育 5 min，溶液又变得浑浊，重复步骤 4。或者加入 200μL 体积的氯仿（37℃），涡旋混匀 10 s，室温 12,000×g 离心 5min。
5. 小心转移上层裂解液至另一个高速离心管内。
6. 加入 0.1 倍体积的 3 M KAc（pH5.2）或 NaAc（pH5.2）和 0.7 倍体积的异丙醇，沉淀质粒 DNA。
7. 8000 rpm 离心 10 min，小心弃上清。
8. 加入 2 mL 70%乙醇，8000 rpm 离心 5min，小心弃上清，室温干燥 10-20min。
9. 加入 **EndoFree Elution Buffer**，重悬质粒 DNA。
注：纯化后的 DNA 可用于内毒素敏感细胞系、原代细胞的转染和显微注射。

Trouble Shooting Guide

Problems	Possible Reasons	Suggested Improvements
Low Yield	Poor Cell lysis.	<ul style="list-style-type: none"> 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).
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 overnight.
Low Yield	Low copy-number plasmid.	Increase culture volume and increase the volume of Buffer A1, B1, N3, and 100% ethanol according to instructions on page 9.
No DNA	Plasmid lost in Host <i>E.coli</i>	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 eluting the plasmid DNA. Re-centrifuge or vacuum again if necessary.

Limited Use and Warranty

This product is intended for *in vitro* research use only. Not for use in human.

This product is warranted to perform as described in its labeling and in BEIWO's literature when used in accordance with instructions. No other warranties of any kind expressed or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by BEIWO. BEIWO's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of BEIWO, to replace the products, BEIWO 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.

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