## Endotoxin Removal Procedure

This protocol is designed to remove endotoxin after the plasmid DNA is purified (Buffers can be scaled up or down accordingly).

**1**. Add **0.2 volume** of **EndoClean buffer** to the plasmid sample in a 1.5 mL sterile tube. (For example, add 0.2 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 °C water bath for about 5 minutes and the solution shall be turbid.

**5.** Centrifuge at top speed at **room temperature** for 5 minutes with the brake off.

**6.** Carefully transfer the upper clear layer solution to 1.5 mL tube. Split in two 1.5 mL tubes, 400- 450 uL each tube.

**7**. Precipitate plasmid DNA with 0.1 volume of 3 M NaAc (pH 5.2) and 2 volume of 100% ethanol.

Note: The EndoClean-treated plasmid DNA is ready transfection of endotoxin sensitive cell lines.



## Contents



## 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.

| Plasmid | Origin | Copy Numbers | Expected Yield (µg per 1 mL) |
|---|---|---|---|
| pSC101 | pSC101 | 5 | 0.1-0.2 |
| pACYC | P15A | 10-12 | 0.4-0.6 |
| pSuperCos | pMB1 | 10-20 | 0.4-1 |
| pBR322 | pMB1 | 15-20 | 0.6-1 |
| pGEM$^{R}$ | Muted pMB1 | 300-400 | 6-7 |
| pBluescript$^{R}$ | ColE1 | 300-500 | 6-8 |
| pUC | Muted pMB1 | 500-700 | 8-12 |

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, JM109, JM110, HB101, TG1 and their derivatives, normally have low plasmid yield. We recommend transform plasmid to a endA- strain if the yield is not satisfactory. For endA+ strains, we recommend use Buffer KB to remove the endonuclease before washing step.

Culture Medium: This procedure is designed for isolating plasmid grown in standard LB medium (Luria Bertani) to 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 cell density 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 F1, B1 and N1.

## 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 TE buffer. The purified DNA is ready for downstream applications such as cloning/subcloning, RFLP, sequencing, and transfection of HEK293 cells. The yield from 1 mL culture is typical around 8 to 12 ug. The mini column has a DNA binding capacity of 40 ug.

## Storage and Stability

Buffer F1 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.

## Kit Contents

| **Catalog Number** | **D1218-01** | **D1218-02** |
|---|---|---|
| Preps | 50 | 250 |
| Lysate Clearance Column | 50 | 250 |
| DNA Columns | 50 | 250 |
| Buffer F1 | 15 mL | 60 mL |
| Buffer F2 | 12 mL | 60 mL |
| Buffer F3 | 12 mL | 60 mL |
| Buffer KB | 25 mL | 125 mL |
| DNA Wash Buffer | 12 mL | 50 mL |
| Elution Buffer | 10 mL | 30 mL |
| RNase A | 50 µL | 210 µL |

*Buffer F3 contains acid acid salts, wear gloves and protective eyewear when handling.
**Add 48 mL (D1218-01) or 200 mL (D1218-02) 96-100% ethanol to DNA Wash Buffer before use.



# Trouble Shooting Guide

| | | |
|---|---|---|
| Low Yield | Poor Cell lysis. | • Resuspend pellet thoroughly by votexing and pipeting prior adding Buffer F2.<br>• Make fresh Buffer F2 if the cap had not been closed tightly. (Buffer F2: 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 (up to 10mL for Minipreps, 200mL for Midipreps, 400 mL for Maxipreps and 3L for Megapreps). Scale up the volume of buffers accordingly. |
| No DNA | Plasmid lost in Host *E.coli* | Prepare fresh culture. |
| Genomic DNA contamination | Over-time incubation after adding Buffer F2. | Do not vortex or mix aggressively after adding Buffer F2. Do not incubate more than 5 minutes after adding solution B1. |
| RNA contamination | RNase A not added to Buffer F1. | Add RNase A to Buffer F1. |
| 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. |

11. **The DNA concentration can be calculated as follows,**

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

**Note:** The DNA is ready for down stream applications such as cloning, sequencing, RFLP or transfection of 293 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.

**Note**: Use less elution buffer if high DNA concentration is desired.



## 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**: Spin down RNase A vial briefly. Add the RNase A solution to Buffer F1 and mix well before use.
- Add 48 mL (1211-01) or 200 mL (D1211-02) 96-100% ethanol to DNA Wash Buffer before use
- Buffer F2 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 F2 after use.
- Ensure the availability of centrifuge capable of 13,000 rpm.
- Carry out all centrifugations at room temperature.
- Buffer F3 contains acid acid, wear gloves and protective eyewear when handling.

## Materials supplied by users:

- 96- 100% ethanol.
- 1.5 mL microcentrifuge tubes
- High speed microcentrifuge.

## EZgene ™ Plasmid Miniprep Protocol

1. Harvest *1-2 mL* of fresh bacterial culture by centrifugation for 30 seconds at 12,000 rpm. Pour off the supernatant and blot the inverted tube on a paper towel to remove residue medium. Remove the residue medium completely.

2. Add 200 µL Buffer F1 and completely resuspend bacterial pellet by vortexing or pipetting (Complete resuspension is critical for optimal yields).

3. Add 200 µL Buffer F2, mix by inverting 10 times (**do not vortex**) and incubate at room temperature for 2 minutes until the solution becomes clear.

4. Add 200 µL Buffer F3 to the sample, mix completely by inverting the vial for 10 times. Incubate at room temperature for 2 min. Transfer the whole lysate to a lysate clearance column.
   Note: If processing more than 2 mL of culture, spin the lysate at 12,000 rpm for 2 min, transfer the relatively clear lysate to a lysate clearance column.

5. Centrifuge at 12,000 rpm for 30 seconds.
   Note: If the lysate still remains in the column, spin for another 30 seconds.

6. Discard the lysate clearance column and add 200 µL 100% ethanol to the flow through in the collection tube, mix well by pipetting and transfer the sample to a DNA mini column.

7. Spin at 12,000 rpm for 30 seconds. Discard the flow through and reuse the collection tube.



8. Optional: Add 500 µL Buffer KB and centrifuge at 12,000 rpm for 30s. Discard the flow-through and put the column back to the collection tube.
   Note: **This step is NOT necessary if the plasmid is being purified from endA- strain such as DH5a and Top 10.** Buffer KB wash is necessary for endA+ strains such as HB101, JM110, JM 101 and their derived strains.

9. Add 500 µL DNA Wash Buffer *(Add ethanol to DNA wash buffer before use)* into the spin column, centrifuge at 12,000 rpm (14,000 - 18,000 x g) for 30 seconds at RT. Remove the spin column from the tube and discard the flow-through.

10. Reinsert the spin column into the collection tube and centrifuge for 1 minute at 13,000 rpm. Note: Residual ethanol will be removed more effectively with the column lid open.

11. Carefully transfer the spin column into an Elution (collection) tube (Provided) and add 50-100 µL Elution Buffer into the column and let it stand for 1 minute. Elute the DNA by centrifugation at 12,000 rpm (14,000-18,000 x g) for 30 seconds to elute DNA.
    Optional: The first elution normally yields around 70% of the plasmid DNA bound to the column. Add the eluted DNA back to the column for another elution yields 20-30% of the DNA bound to the column.