

# Mobius™ and UltraMobius™ 200 Plasmid Kits



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# Mobius™ and UltraMobius™ 200 Plasmid Kits

## About the Kit

Mobius 200 Plasmid Kit	25 rxn	70970-3
UltraMobius™ 200 Plasmid Kit	25 rxn	71090-3

## Description

The Mobius 200 Plasmid Kits are designed for convenient, rapid isolation of high purity plasmid DNA suitable for the most demanding applications. The method features anion exchange chromatography with a patented polymer-based resin and produces plasmid DNA that can be used for transfection, sequencing and all enzymatic procedures. The kit incorporates a novel filtration device that minimizes the centrifugation steps required for the clarification of bacterial lysates. Following preparation of the clarified lysate, plasmid is purified using a pre-packed Mobius 200 Column and the optimized buffers supplied with the kit. Eluted plasmid DNA is concentrated and desalted by precipitation with isopropanol. The procedure uses no organic extractions and can be completed in less than 1 hour.

Mobius 200 Columns have a DNA binding capacity of over 200 µg of plasmid DNA. The standard protocol is designed to process 35 ml of an overnight bacterial culture for high and low-copy number plasmids. The typical yields for high-copy number plasmids are > 200 µg DNA. For low-copy number plasmids, the yield is 30–60 µg DNA.

For the majority of plasmid DNA applications, such as routine transfection, cloning, sequencing and transformation of competent *E. coli*, the standard Mobius 200 Plasmid Kit provides DNA with sufficiently high purity and very low endotoxin levels (< 500 EU/mg). In applications where extremely low endotoxin levels are required, for example in microinjection and transfection of endotoxin-sensitive cell lines, the UltraMobius 200 Plasmid Kit is recommended. The UltraMobius 200 Plasmid Kit uses all of the same components as the Mobius 200 Plasmid Kit, plus an additional reagent to reduce endotoxin levels below 20 EU/mg plasmid DNA.

## Components

- 25 Mobius 200 Columns
- 25 Mobius 200 Filters
- 1 ml RNase A Solution
- Mobius Buffer Kit
  - 1 ml RNase A Solution
  - 100 ml Bacterial Resuspension Buffer (1)
  - 100 ml Bacterial Lysis Buffer (2)
  - 100 ml Mobius Neutralization Buffer (3)
  - 30 ml Detox Agent (UltraMobius only) (4)
  - 125 ml Mobius Equilibration Buffer (A)
  - 250 ml Mobius Wash Buffer (B)
  - 65 ml Mobius Elution Buffer (C)
  - 12.5 ml TE Buffer

## Storage

Store all components of the kit at room temperature (21–25°C). After combining the RNase A and Bacterial Resuspension Buffer, this solution should be stored at 4°C.

## Required equipment and reagents

- Isopropanol
- Ethanol (70%) prepared with nuclease-free sterile water
- High-speed centrifuge compatible with 50 ml conical tubes and 30 ml polycarbonate tubes
- Sterile 2 and 5 ml pipets
- Sterile 15 ml conical centrifuge tubes
- Sterile water
- Microcentrifuge (optional)



## Mobius 200 and UltraMobius™ 200 Protocol

### 35 ml culture (high-copy or low-copy number plasmid)

This protocol is intended for routine purification of high- and low-copy number plasmids. The yields for high-copy number plasmids typically exceed 200 µg. For low-copy number plasmids, the yields are typically around 30–60 µg.

**Note:** *Prior to the first use of the kit:*

**A. Add 1 ml (the entire volume) RNase A solution to the Bacterial Resuspension Buffer (bottle 1).** Spin the tube in a microcentrifuge briefly to force the contents to the bottom of the tube and transfer the entire volume to a bottle of Resuspension Buffer using a sterile pipet. After addition of the RNase, mark the box on the bottle as a reminder and store the reagent at 4°C.

**Before starting a plasmid isolation:**

**B. Check the Bacterial Lysis Buffer (bottle 2) for precipitation.** If a precipitate is observed, warm the bottle briefly at 30–40°C until the precipitate dissolves. Lysis buffer contains Sodium Hydroxide! Wear gloves, lab coat and protective eyewear. Clean any exposed skin thoroughly with soap and water. If exposure to the eye occurs, rinse eyes with copious amounts of water.

**C. Chill the Mobius Neutralization Buffer (bottle 3) on ice for at least 10 minutes prior to use.**

### Culture growth

1. From a freshly streaked plate, transfer a single bacterial colony to 5 ml LB broth containing the appropriate antibiotic (see page 8 for preparation). Incubate at 37°C for 8 h in a shaking incubator at 300 rpm.
2. Transfer a few microliters of this culture into 35 ml sterile LB broth containing antibiotic in an appropriately sized flask. Incubate at 37°C for 12–16 h in a shaking incubator at 300 rpm. The OD<sub>600</sub> at harvest should be approximately 3–5 when using sterile medium as a spectrophotometer blank.
3. Harvest the cells by centrifugation at 5000 × g for 10 min. Carefully decant the supernatant and hold the tube, inverted, for several seconds to remove residual medium. Blot the mouth of the inverted tube on a paper towel to remove any residual medium. It is convenient to decant into a bleach solution to sterilize the supernatant prior to disposal.

**Note:** *The use of 50 ml conical tubes is recommended for cell harvesting, lysis and initial clarification. It is important to use a tube with sufficient capacity to allow adequate mixing during resuspension, lysis and neutralization steps. The use of a smaller tube can reduce plasmid yield.*

### Preparation of cleared lysate

1. Be sure the Neutralization Buffer (bottle 3) is chilled on ice for 10 min before beginning.
2. Resuspend the bacterial pellet in 2.1 ml of Bacterial Resuspension Buffer (bottle 1). Pipet up and down and/or gently vortex until there are no visible clumps and the pellet is completely resuspended. Failure to fully resuspend the bacterial pellet can reduce plasmid yield.
3. Add 2.1 ml of Bacterial Lysis Buffer (bottle 2) to the fully resuspended bacteria. Recap the tubes and tip or swirl gently but firmly to mix, until the lysate appears translucent and viscous. **Do not vortex!** This can cause shearing of genomic DNA, resulting in genomic contamination and reduced plasmid yield. Incubate the lysate at room temperature for 5 min.

**Notes:** *Gentle but complete mixing is critical for maximum plasmid yields. The resulting lysate should appear nearly clear although viscous and bubbly. "Pockets" of unlysed cells within the mixture show a stringy, opaque appearance and signify insufficient mixing, which can contribute to reduced plasmid yields. Do not exceed the recommended 5 min incubation. Excessive exposure to alkaline conditions can contribute to nicking and irreversible denaturation of plasmid DNA.*

*Keep the Bacterial Lysis Buffer tightly capped when not in use. Atmospheric CO<sub>2</sub> will lessen the effectiveness of the buffer.*



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4. Add 2.1 ml chilled Mobius Neutralization Buffer (bottle 3), recap, and mix thoroughly by inversion to form a uniform flocculent precipitate. The mixture should become less viscous as genomic DNA, protein, detergent and cell debris are precipitated. Incubate the mixture on ice for 5 min.

*Note:* The properly mixed neutralized lysate should have two components: white flocculent precipitate and the reduced viscosity solution. The presence of stringy, viscous debris within the neutralized lysate indicates poor mixing which can lead to reduced plasmid yield.

5. During the incubation, remove the upper and lower caps of a Mobius 200 Column, decant excess storage buffer, and place the column in a 15 ml conical tube. Add 5 ml of Mobius Equilibration Buffer (bottle A) into the column reservoir and verify that the column is flowing by gravity. The flow of buffer may take a few moments to begin. Allow the entire 5 ml volume to flow through the column. The column will not run dry because the flow will cease when the buffer meniscus reaches the top frit of the column.
6. Centrifuge the neutralized lysate at  $10,000 \times g$  for 2 min (room temperature or  $4^{\circ}\text{C}$ ) to remove the bulk of the insoluble material. Insert a Mobius Filter unit into a 15 ml conical tube and **slowly** decant the cleared lysate supernatant into it.

*Notes:* The amount of clarified lysate may just exceed the Mobius Filter reservoir capacity. The filter will begin to flow immediately upon adding the lysate and slow addition should allow the user to decant all of the clarified lysate without stopping.

*For agarose gel analysis (optional, see page 6), remove a 25  $\mu\text{l}$  (for high-copy) or 75  $\mu\text{l}$  (for low-copy) sample of the clarified lysate. Maintain the fraction on ice or store at  $-20^{\circ}\text{C}$ .*

*Tip:* The clarified, neutralized lysate can be stored frozen at  $-20^{\circ}\text{C}$  or  $-70^{\circ}\text{C}$  for later processing, if desired. Frozen lysates must be clarified after thawing by centrifugation ( $10,000 \times g$  for 10 min) prior to loading the Mobius 200 Column to remove any additional precipitate that may have formed.

7. **UltraMobius™ 200 Plasmid Kit only:** Add 1 ml Detox Agent (bottle 4) to the cleared lysate, mix gently and incubate on ice for 15 min.
8. **UltraMobius 200 Plasmid Kit only:** Centrifuge the Detox Agent treated lysate for 10 min at  $10,000 \times g$ .

## Mobius 200 Column chromatography

1. Place the equilibrated Mobius 200 Column in a new 15 ml conical tube and transfer the entire clarified lysate volume to the column reservoir. Allow the entire volume to flow through the column by gravity. The column does not need to be attended during this period.
2. Transfer the Mobius 200 column to a new 15 ml conical tube. Add 5 ml of Mobius Wash Buffer (bottle B) to the column reservoir and allow the entire volume to flow through the column by gravity.

*Note:* For agarose gel analysis (optional, see page 5), remove a 25  $\mu\text{l}$  (for high-copy) or 75  $\mu\text{l}$  (for low-copy) sample of the flow through and a 25  $\mu\text{l}$  (for high-copy) or 75  $\mu\text{l}$  (for low-copy) sample of the wash. Maintain the fractions on ice or store at  $-20^{\circ}\text{C}$ .

3. Transfer the loaded Mobius 200 column to a new 15 ml conical tube and elute the plasmid DNA by adding 2 ml of Mobius Elution Buffer (bottle C) to the reservoir. Allow the entire volume to flow through the column by gravity. Collect the entire volume in a single fraction.

*Optional Stopping Point:* The eluted DNA may be stored at  $4^{\circ}\text{C}$  and precipitated the following day.

*Note:* For agarose gel analysis (optional, see page 5), remove a 10  $\mu\text{l}$  (for high-copy) or 30  $\mu\text{l}$  (for low-copy) sample of the eluate.



## Precipitation of plasmid DNA

1. Remove the column from the tube and transfer the entire volume of the eluted sample into a 30 ml polycarbonate centrifuge tube. Add 1.4 ml of isopropanol to the eluted sample to precipitate the plasmid DNA. Mix gently and immediately centrifuge at  $15,000 \times g$  for 20 min (room temperature or  $4^{\circ}\text{C}$ ).  
Alternatively, transfer 1 ml portions of the eluted plasmid DNA into 2 sterile 1.6–2 ml microcentrifuge tubes. Add 0.7 ml of isopropanol to each tube, mix gently and centrifuge immediately at  $15,000 \times g$  for 15 min (room temperature or  $4^{\circ}\text{C}$ ).
2. Carefully aspirate and discard the supernatant, avoiding contact with the tube wall. It may be helpful to mark the exterior position of the tubes with a lab marker to aid in locating the DNA pellet. Isopropanol-precipitated DNA pellets are often diffuse, translucent, and spread over a large portion of the interior tube wall. Addition of Pellet Paint® (Cat. No. 69049-3) or Pellet Paint NF (Cat. No. 70748-3) Co-Precipitant prior to the addition of isopropanol can aid in visualizing the precipitated material.

*Note:* Pellet Paint® and Pellet Paint NF Co-Precipitant have not been extensively tested for compatibility with DNA transfection or DNA microinjection into eukaryotic cells.

3. Wash the DNA pellet by adding 2 ml of 70% ethanol (room temperature) and swirling the tube gently. Microcentrifuge option: Wash the DNA pellets by adding 1 ml each 70% ethanol and invert the tubes gently. Recentrifuge if the pellet becomes dislodged during washing.

*Caution:* For ultra-low endotoxin levels, the 70% ethanol must be prepared with endotoxin-free water.

4. Centrifuge at  $15,000 \times g$  for 10 min (room temperature or  $4^{\circ}\text{C}$ ).
5. Decant the supernatant and invert the tube(s) on a clean paper towel to remove residual ethanol.
6. Air or vacuum-dry the pellet until the visible liquid has evaporated. Avoid excessive drying, which can make dissolving the plasmid DNA more difficult. Dissolve the pellet in a total volume of 0.05–0.25 ml of TE Buffer or nuclease-free water. To ensure complete recovery, use two or three successive aliquots of TE or water and use a pipet to break up the pellet while washing the tube walls. Transfer the plasmid solution to a clean, labeled 1.5 ml microcentrifuge tube and store at  $-20^{\circ}\text{C}$ .

*Caution:* For ultra-low endotoxin levels, the TE or water must be endotoxin-free.

## Determination of Plasmid Yield and Purity

### UV absorbance measurements

DNA yield can be determined spectrophotometrically by measuring the absorbance of a dilution of the purified plasmid at 260 nm. The normal calculation for dsDNA is  $A_{260} = 1.0 = 50 \mu\text{g/ml}$ , for a 1 cm path length cuvette. Assuming a plasmid yield of 200  $\mu\text{g}$  resuspended in 0.2 ml, the expected absorbance of an undiluted sample will be approximately  $A_{260} = 20$ . For optimal accuracy, the target absorbance for UV spectrophotometer readings should be between 0.1–0.5. Therefore, an aliquot of the plasmid DNA should be diluted from 1:40 to 1:200 in deionized water prior to measuring UV absorbance. The recorded  $A_{260}$  should be multiplied by the dilution and the extinction coefficient ( $50 \mu\text{g/ml}/1.0 A_{260}$ ) to obtain the concentration of plasmid DNA in  $\mu\text{g/ml}$ .

Note that the results of  $A_{260}$  readings are accurate only in the absence of significant contamination by RNA and other UV-absorbing materials. Critical samples should also be analyzed by agarose gel analysis to confirm that the UV absorbing material is, in fact, plasmid and that the absorbance reading is consistent with the yield observed on an agarose gel.

Additional information about purity can be obtained by reading the absorbance at 280 nm and determining the  $A_{260}/A_{280}$  ratio. Nucleic acids have an average absorbance maximum of approximately 260 nm, whereas proteins (assuming a normal distribution of aromatic residues) have an average absorbance maximum of 280 nm. Pure DNA and typical Mobius plasmid isolates have  $A_{260}/A_{280}$  ratio of 1.75–1.95. Preparations contaminated with protein have significantly lower ratios of 1.3–1.5, while higher ratios (greater than or equal to 2.0) may indicate the presence of significant levels of RNA.



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## Agarose gel analysis

Fractions from the purification of plasmids using the Mobius Kits can be analyzed by agarose gel electrophoresis. Prior to gel analysis, samples must be precipitated with isopropanol to remove salts that interfere with electrophoresis. The recommended sample volumes in the table below represent approximately 0.5% (high-copy number plasmids) and 1.5 % (low-copy number plasmids) of the total volume of the fraction being analyzed. The small amounts of DNA present (about 1 µg) in each pellet makes the precipitated DNA invisible within the tube. To avoid the loss of samples during handling, add Pellet Paint® or Pellet Paint NF Co-Precipitant to each sample prior to precipitation (see step 3, below). Pellet Paint® Co-Precipitant allows the precipitated material to be easily located on the tube wall and can be used to track the DNA pellet during washing and to confirm complete resuspension.

## Preparation of Mobius and UltraMobius™ purification fractions for gel analysis

1. Consult the tables below for either high- or low-copy number plasmid to determine the appropriate volumes. Pipet the indicated volume of each of the fractions into clean microcentrifuge tubes.
2. Add water to each fraction to 100 µl total volume. The required volumes of water are indicated below.
3. (optional): If using Pellet Paint® Co-Precipitant, add 2 µl to each fraction.
4. Add 100 µl isopropanol to each fraction, mix well, and centrifuge 15 min in a microcentrifuge at maximum speed.
5. Carefully decant or aspirate the supernatant and wash the pellet with 0.5 ml 70% ethanol. Spin at maximum speed for 3 min.
6. Carefully remove the supernatant and dry the sample pellets. Placing the open tubes in a heat block at 50–70°C or in a centrifugal vacuum unit can accelerate drying.
7. Resuspend each pellet in 10 µl TE buffer.
8. Add 2 µl of 6X DNA Gel Loading Buffer (Cat. No. 69046-3) to each tube.
9. Load 6 µl of each sample on a 0.8–1% agarose gel containing ethidium bromide.

High-copy number plasmid – Standard Protocol				
Fraction	Sample volume	Additional water	Resuspend	Gel load volume
Mobius Filter Lysate	25 µl	75 µl	10 µl	6 µl
Flow-through	25 µl	75 µl	10 µl	6 µl
Wash	25 µl	75 µl	10 µl	6 µl
Elution	10 µl	90 µl	10 µl	6 µl

  

Low-copy number plasmid – Standard Protocol				
Fraction	Sample volume	Additional water	Resuspend	Gel load volume
Mobius Filter Lysate	75 µl	25 µl	10 µl	6 µl
Flow-through	75 µl	25 µl	10 µl	6 µl
Wash	75 µl	25 µl	10 µl	6 µl
Elution	30 µl	70 µl	10 µl	6 µl



## Additional Notes

### Plasmids and host strains

The Mobius 200 Plasmid Kits can be used to purify a variety of plasmids\* from many different *E. coli* host strains. Most commonly used plasmids fall into either high-copy number (> 200 copies per cell) or low-copy number (10–40 copies per cell) categories depending on the plasmid replicon. The term “mid-copy” has also been applied to plasmid having between 10 and 40 copies per cell, to distinguish them from plasmids maintained at very low copy numbers (e.g. pETcoco™ vectors). Here we refer to the pETcoco plasmids as “single-copy” when maintained in the presence of 0.2% glucose, and “low-copy” when copy number is amplified to ~40 copies/cell in the presence of 0.01% arabinose. The Mobius 200 protocol uses 35 ml of culture. For higher yields of low-copy number plasmids, a larger volume of bacterial culture may be processed. The table below classifies some commonly used plasmids according to copy number.

\* The purification of long chain nucleic acids (> 20 kbp) by anion exchange chromatography is protected by EP 0 268 946 and corresponding patents. Purchase of the Mobius Kits does not include a license under these patents.

Plasmid series	Classification
pACYC	<b>Low-copy</b>
pACYCDuet	<b>Low-copy</b>
pBAC™, pBACgus	High-copy
pBluescript®	High-copy
pBR322	<b>Low-copy</b>
pCITE®	High-copy
pET	<b>Low-copy</b>
pETBlue™	High-copy
pETcoco™	<b>Low-copy*</b>
pETDuet™	<b>Low-copy</b>
pGEM®	High-copy
pLysS, pLysE, pLacI	<b>Low-copy</b>
pSCREEN™	High-copy
pSTBlue	High-copy
pT7Blue	High-copy
pTZ	High-copy
pTriEx™	High-copy
pUC	High-copy

\*The single-copy pETcoco plasmid must be amplified to a low-copy plasmid using arabinose to prior to plasmid purification. See Technical Bulletin 333.

Although most host strains can be used successfully with the Mobius method, the quality of the plasmid DNA can vary based on specific hosts used. Novagen’s NovaBlue produces very high quality plasmid and has been used as the standard to qualify endotoxin levels with the Mobius Kits. Useful mutations for plasmid preparation affect the genes *endA* (eliminates endonuclease I activity, which improves the quality of miniprep plasmid) and *recA* (eliminates general recombination and inhibits the formation of plasmid multimers).

Symptoms of host-related problems include impurities due to excess carbohydrates that can inhibit some enzymatic procedures, excess nicked plasmid due to high endonuclease levels, and difficulty in restriction digestion and/or transformation of restriction-plus hosts due to incompatible modification of the DNA. In these cases it may be worthwhile to transform the plasmid into another host such as DH1 or NovaBlue. The NovaBlue strain (genotype: *endA1 hsdR17(r<sub>K12</sub><sup>-</sup> m<sub>K12</sub><sup>+</sup>) supE44 thi-1 recA1 gyrA96 relA1 lac F' [proA<sup>+</sup> B<sup>+</sup> lacI<sup>r</sup> ZΔM15 ::Tn10(Tc<sup>R</sup>)]*) is



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available from Novagen as competent cells, Singles™ Competent Cells, HT96™ competent cells or as a glycerol stock.

## Culture conditions

Culture conditions can dramatically affect plasmid yield and quality. The Mobius procedure has been optimized for cultures in LB broth (see recipe below). Richer media such as Terrific Broth may produce more cell mass, but the yield and quality of plasmid may suffer due to greater levels of cellular and media components that can interfere with plasmid binding and separation on the anion exchange resin.

**Preparation of LB broth:** Per liter, combine 10 g tryptone, 5 g yeast extract and 10 g NaCl. Add deionized water to ~900 ml and dissolve with stirring. Adjust the pH to 7.5 with 1 N NaOH, make up to 1 liter with water, and autoclave. Cool to 60°C before adding the appropriate antibiotic (e.g. 50 µg/ml carbenicillin).

Cultures should always be grown in the presence of appropriate antibiotic to maintain selection of the plasmid. Particular care should be taken with the ampicillin-resistance marker β-lactamase, which is present on many commonly used plasmids. During growth, cells secrete β-lactamase into the medium where it can rapidly degrade the antibiotic. Degradation of ampicillin is also enhanced during the late stages of cell growth when the pH of the culture drops. This effect can be alleviated by using carbenicillin (Cat. No. 69101-3) instead of ampicillin, since carbenicillin is less susceptible to degradation under acidic conditions.

Starter cultures for plasmid isolation are prepared by inoculating 2–5 ml LB medium containing antibiotic from freshly streaked, well-isolated colonies grown on LB agar plates containing antibiotic. After shaking at 250–300 rpm 37°C for 8 h, dilute the cells 1:200–1:500 into a culture flask containing prewarmed LB broth containing antibiotic and shake at 37°C for 12–16 h. For proper aeration use a culture flask that has a volume at least 4-fold greater than the culture volume (e.g., a 500 ml Erlenmeyer flask for a 100 ml culture). Flasks intended for bacterial growth (e.g. Fernbach flasks) or flasks with baffles are also suitable, although not required.

As a matter of routine, glycerol stocks should be prepared from cultures grown from single colonies to an OD<sub>600</sub> of ~0.5 to avoid the overgrowth of non-plasmid bearing cells.

## Amplification of low-copy number plasmids

Although the Mobius 200 Kits provide a reproducible method for purification of low-copy number plasmids grown in LB broth without amplification, it is possible to increase the copy number of ColE1-derived plasmids through manipulation of culture conditions, which may increase the yield of difficult plasmids. Plasmids such as pBR322 can be induced to increase their copy number through the addition of chloramphenicol to the growth medium, which inhibits host protein synthesis while plasmid replication continues (Clewell and Helinski, 1972). Although this phenomenon is not clearly understood, historically investigators added 170 µg/ml chloramphenicol to cultures in log phase (OD<sub>600</sub> = 0.4) followed by 12–16 h additional incubation prior to harvest (Maniatis et al., 1982). More recently, superior amplification of pBR322-type plasmids was reported using 10–20 µg/ml chloramphenicol to partially inhibit protein synthesis (Frenkel and Bremer, 1986); in this case amplification appeared to depend on the *relA* gene product. Other strategies such as amino acid starvation and growth temperature have also been reported to cause plasmid amplification (Hecker et al., 1988; Riethdorf et al., 1989; Hofmann et al., 1990). Protocols for the amplification of low-copy number plasmids must be developed empirically under the desired growth conditions. For evaluation of plasmid amplification with a specific vector/host combination, the recommended conditions are growth in LB to an OD<sub>600</sub> of 1–2, addition of 10–20 µg chloramphenicol/ml culture volume, and 12–16 h incubation at 37°C. Note that the NovaBlue strain carries the *relA1* mutation, so is unsuitable for amplification by this method.



## Bacterial endotoxins

Endotoxins, also referred to as lipopolysaccharide (LPS), an essential component of the outer membrane of gram-negative cells such as *Escherichia coli* and *Salmonella typhimurium*, are present at about  $3.5 \times 10^6$  copies/cell (Nikaido and Vaara, 1987). In wild-type *E. coli*, the LPS molecule is composed of three basic components: an O polysaccharide side-chain, a core oligosaccharide and Lipid A. The amphipathic nature of the LPS molecule allows it to form micelles as well as embed itself in the outer membrane, maintaining the hydrophilic, negatively charged O and core polysaccharides on the exterior. In the commonly used *E. coli* strains, K-12 and B, the O polysaccharide is absent. B strains have an additional deletion of the core polysaccharide. During the lysis of bacterial cells with detergent, LPS and other membrane components are solubilized. Since LPS has a natural affinity for silica surfaces, the isolation of plasmids using a silica-based solid phase can often result in the co-purification of endotoxin. Other purification procedures such as CsCl banding and standard alkaline lysis can also result in the co-purification of endotoxins. Endotoxin contamination in plasmid isolates can cause cytotoxicity in transfection and microinjection procedures. (Cotton et al., 1994).

In contrast to other commercial plasmid purification media which use a silica matrix, the Mobius 200 Columns are packed with a highly hydrophilic ion exchange bead that allows plasmid DNA to be selectively purified without co-purification of LPS. The result is that plasmids isolated with the standard Mobius 200 Plasmid Kits are substantially lower in LPS level than isolates from other kits as measured by the *Limulus* amoebocyte lysate assay (Hendriks et al., 2000).

## Lowest endotoxin levels with UltraMobius™ 200 Plasmid Kit

The UltraMobius 200 Plasmid Kit combines the Mobius 200 Columns with Detox Agent treatment, resulting in exceptionally low levels of endotoxin contamination. The UltraMobius protocol is nearly identical to the standard plasmid isolation procedure, requiring only the addition of Detox Agent to the cleared lysate and a brief (15 min) incubation on ice prior to chromatography. Remember to use fresh sterile disposable centrifuge tubes, and use endotoxin-free water for making the 70% EtOH and buffer for resuspending the final DNA pellet. Endotoxin contamination can occur if glassware or solutions are sterilized in an autoclave previously used for killing bacterial cultures; therefore, it is best to avoid using autoclaved glassware and solutions.



## Troubleshooting Guide

Problem	Probable cause	Solution
Low plasmid yield	Low plasmid copy number	Try plasmid amplification with chloramphenicol as described on page 8.
	Insufficient cell pellet resuspension	Repeat isolation, being careful to vortex until all visible clumps are gone.
	Inadequate lysis	Be certain that Bacterial Lysis Buffer and cell suspension are fully mixed to facilitate complete lysis of bacterial cells. Ensure that the Bacterial Lysis Buffer is tightly capped when not in use.
Plasmid in wash fraction	Evaporation of Wash Buffer resulting in excessive salt concentration and plasmid elution during wash	Replace Mobius Wash Buffer. Close bottle lid tightly after use to avoid evaporation.
Plasmid contaminated with RNA	RNase A not added to Resuspension Buffer	Repeat procedure, add RNase A to Bacterial Resuspension Buffer prior to use. Store buffer at 4°C after use.
Plasmid contaminated with genomic DNA	Excessive mixing during lysis and neutralization steps	Repeat procedure. Be more gentle when mixing fractions during lysis and neutralization steps
Slow column flow rates	High cell mass contributing to clogging of column	Reduce cell culture volume or increase centrifugation time to 30 min at 15,000 × g to pellet fine precipitates prior to chromatography.

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