Mobius™ and UltraMobius™ 200 Plasmid Kits

Table of Contents

About the Kits 2
- Description 2
- Components 2
- Storage 2
- Required equipment and reagents 3

Mobius 200 and UltraMobius 200 Protocol 3
- 35-ml culture (high-copy or low-copy number plasmid) 3
  - Culture growth 3
  - Preparation of cleared lysate 4
  - Mobius 200 Column chromatography 5
  - Precipitation of plasmid DNA 5

Determination of Plasmid Yield and Purity 6
- UV absorbance measurements 6
- Agarose gel analysis 6
- Preparation of Mobius and UltraMobius purification fractions for gel analysis 7

Troubleshooting Guide 8

Additional Notes 8
- Plasmids and host strains 9
- Culture conditions 10
  - Amplification of low-copy number plasmids 10
- Bacterial endotoxins 11
- Lowest endotoxin levels with UltraMobius 200 Plasmid Kit 11

References 11

USA and Canada
Tel (800) 526-7319
novatech@novagen.com

Germany
Tel 0800 100 3496
techservice@merckbiosciences.de

United Kingdom and Ireland
Freefone 0800 622935
Toll Free 1800 409445
customer.service@
merckbiosciences.co.uk

All Other Countries
Contact Your Local Distributor
www.novagen.com
novatech@novagen.com

A Brand of EMD Biosciences, Inc., an Affiliate of Merck KGaA, Darmstadt, Germany
www.novagen.com

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About the Kits

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 fast, convenient, and economical isolation of > 200 µg ultrapure plasmid DNA from 35-ml overnight bacterial cultures using high-copy number plasmids (> 30 µg for low-copy number plasmids). Based on an alkaline lysis procedure combined with streamlined anion exchange chromatography, the Mobius method produces plasmid DNA suitable for all molecular biology applications, including restriction analysis, *in vitro* transcription, sequencing, and transfection.

The Mobius 200 Plasmid Kit features the same Fractogel® high-capacity anion-exchange tentacle resin as the other Mobius kits, and incorporates a unique filter basket that minimizes the centrifugation steps required for the clarification of bacterial lysates.

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.

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, such as microinjection and transfection of endotoxin-sensitive cell lines, the UltraMobius 200 Plasmid Kit is recommended. The UltraMobius 200 Plasmid Kit uses 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
- 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, the 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 and microcentrifuge tubes (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. Yields for high-copy number plasmids typically exceed 200 µg. For low-copy number plasmids, the yields are typically 30–60 µg.

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

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

Before starting a plasmid isolation:

B. Examine Bacterial Lysis Buffer (bottle 2) for precipitation. If precipitate is observed, warm bottle briefly at 30–40°C until 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 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 appropriate antibiotic (see page 10 for preparation). Incubate at 37°C for 8 h in shaking incubator at 300 rpm.
2. Transfer a few microliters of this culture to 35-ml sterile LB broth containing antibiotic in an appropriately sized flask. Incubate at 37°C for 12–16 h in shaking incubator at 300 rpm. The OD_{600} at harvest should be approximately 3–5 when using sterile medium as a spectrophotometer blank.
3. Harvest cells by centrifugation at 5000 × g for 10 min. Carefully decant supernatant and hold tube, inverted, for several seconds to drain residual medium. Blot mouth of inverted tube on paper towel to remove any residual medium. It is convenient to decant into a bleach solution to sterilize the supernatant before disposal.

Note: *The use of 50-ml conical tubes is recommended for cell harvest, 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. Chill Neutralization Buffer (bottle 3) on ice for 10 min.
2. Resuspend bacterial pellet in 2.1 ml Bacterial Resuspension Buffer (bottle 1). Pipet up and down, and/or gently vortex until there are no visible clumps and pellet is completely resuspended. Failure to fully resuspend bacterial pellet can reduce plasmid yield.
3. Add 2.1 ml Bacterial Lysis Buffer (bottle 2) to the fully resuspended bacteria. Recap tubes. Tip tubes, or swirl gently but firmly to mix, until lysate appears translucent and viscous. **Do not vortex!** Vortexing can cause shearing of genomic DNA, resulting in genomic contamination and reduced plasmid yield. Incubate lysate at room temperature for 5 min.

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

Keep Bacterial Lysis Buffer tightly capped when not in use. Atmospheric CO_2 will decrease the effectiveness of the buffer.

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

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

5. During incubation, remove upper and lower caps of a Mobius 200 Column. Decant excess storage buffer. Place column in 15-ml conical tube. Add 5 ml Mobius Equilibration Buffer (bottle A) into column reservoir and verify "gravity flow," which may take a few moments to begin. Allow entire 5-ml volume to flow through column. The column will not run dry because flow will cease when buffer meniscus reaches the top frit of column.
6. Centrifuge neutralized lysate at 10,000 × g for 2 min (room temperature or 4°C) to remove the bulk of insoluble material. Insert a Mobius Filter unit into 15-ml conical tube. **Slowly** decant cleared lysate supernatant into tube.

Notes: *The volume of clarified lysate may slightly exceed the Mobius Filter reservoir capacity. Flow through the filter will begin immediately upon addition of the lysate; slow addition should allow the user to decant all the clarified lysate without interruption.*

For agarose gel analysis (optional, see page 6), remove 25 µl (for high-copy) or 75 µl (for low-copy) sample of clarified lysate. Maintain fraction on ice, or store at –20°C.

Tip: *The clarified, neutralized lysate can be frozen at –20°C or –70°C and stored for later processing, if desired. To remove any precipitate that may have formed, frozen lysates must be clarified after thawing by centrifugation (10,000 × g for 10 min) prior to loading Mobius 200 Column.*

7. **UltraMobius™ 200 Plasmid Kit only**: Add 1 ml Detox Agent (bottle 4) to cleared lysate. Mix gently. Incubate on ice for 15 min.
8. **UltraMobius 200 Plasmid Kit only**: Centrifuge Detox Agent treated lysate for 10 min at 10,000 × g.

Mobius™ 200 Column chromatography

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

Note: For agarose gel analysis (optional, see page 5), remove 25 µl (for high-copy) or 75 µl (for low-copy) sample of flow through and 25 µl (for high-copy) or 75 µl (for low-copy) sample of wash. Maintain fractions on ice, or store at –20°C.

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

Optional Stopping Point: The eluted DNA may be stored overnight at 4°C and precipitated the following day.

Note: For agarose gel analysis (optional, see page 6), remove 10 µl (for high-copy) or 30 µl (for low-copy) sample of eluate.

Precipitation of plasmid DNA

1. Remove column from tube and transfer entire volume of eluted sample into 30-ml polycarbonate centrifuge tube. Add 1.4 ml isopropanol to eluted sample to precipitate plasmid DNA. Mix gently and immediately centrifuge at 15,000 × g for 20 min (room temperature or 4°C).
   Alternatively, transfer 1-ml portions of eluted plasmid DNA into 2 sterile 1.6–2 ml-microcentrifuge tubes. Add 0.7 ml isopropanol to each tube. Mix gently and centrifuge immediately at 15,000 × g for 15 min (room temperature or 4°C).
2. Carefully aspirate and discard supernatant, avoiding contact with tube wall. To aid in locating the DNA pellet, it may be helpful to mark the exterior position of the tubes with permanent marking pen. 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 DNA pellet by adding 2 ml 70% ethanol (room temperature) and swirling tube gently. Microcentrifuge option: Wash DNA pellets by adding 1 ml 70% ethanol and invert tubes gently. If pellet becomes dislodged during washing, recentrifuge.

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

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

Caution: For ultra-low endotoxin levels, 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 µg/ml for a cuvette with a 1-cm path length. Assuming a plasmid yield of 200 µg 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 volume of the plasmid DNA should be diluted from 1:40 to 1:200 with deionized water before measuring UV absorbance. To obtain the concentration of plasmid DNA in µg/ml, multiply the recorded A_{260} by the dilution factor and the extinction coefficient (50 µg/ml/1.0 A_{260}).

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.

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 Table 1 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 make 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, page 7). 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 Table 1 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 6X DNA Gel Loading Buffer (Cat. No. 69046-3) to each tube.
9. Load 6 µl of each sample on an 0.8–1% agarose gel containing ethidium bromide.

Table 1

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

Troubleshooting Guide

Table 2

Problem	Probable cause	Solution
Low plasmid yield	Low plasmid copy number	Try plasmid amplification with chloramphenicol, as described on page 10.
	Insufficient cell pellet resuspension	Repeat isolation. Vortex until all visible clumps are gone.
	Inadequate lysis	Fully mix Bacterial Lysis Buffer and cell suspension 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.

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 approximately 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. Table 3 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. The use of the pETcoco vectors is covered under U.S. Patent No. 5,874,259 and other patents pending. This product is sold under license from the University of Wisconsin–Madison and is limited solely for research purposes.

Table 3

Plasmid series	Copy Number	Protocol
pACYC	10–12	Low-copy
pACYCDuet™	10–12	Low-copy
pBAC™, pBACgus	> 500	High-copy
pBluescript®	> 500	High-copy
pBR322	15-20	Low-copy
pCDF, pCDFDuet™	20-40	Low-copy
pCITE®	> 500	High-copy
pCOLADuet™	~40	Low-copy
pET	~40	Low-copy
pETBlue™	> 500	High-copy
pETcoco™	~40*	Low-copy*
pETDuet™	~40	Low-copy
pGEM®	> 500	High-copy
pLysS, pLysE, pLacI	10–12	Low-copy
pRSF, pRSFDuet™	> 200	High-copy
pSCREEN™	> 500	High-copy
pSTBlue	> 500	High-copy
pT7Blue	> 500	High-copy
pTriEx™	> 500	High-copy
pUC	> 500	High-copy

*The single-copy pETcoco plasmid must be amplified to a low-copy plasmid using arabinose to prior to plasmid purification. See User Protocol TB 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. The Novagen NovaBlue strain 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 plasmid DNA) 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_{K12}^- m_{K12}^+) *supE44 thi-1 recA1 gyrA96 relA1 lac* F' [*proA*$^+$*B*$^+$ *lacI*^{q}*ZΔM15* ::Tn*10*(Tet^R)]) is available from Novagen as competent cells, Singles™ Competent Cells, GigaSingles™ 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 approximately 900 ml and dissolve with stirring. Adjust the pH to 7.5 with 1 N NaOH, adjust volume to 1 L 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 four-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_{600} of approximately 0.5 to avoid the overgrowth of non-plasmid bearing cells. Specific instructions for the storage of strains can be found in User Protocol TB055.

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 (1). Although this phenomenon is not clearly understood, historically investigators added 170 µg/ml chloramphenicol to cultures in log phase (OD_{600} = 0.4) followed by 12–16 h additional incubation prior to harvest (2). More recently, superior amplification of pBR322-type plasmids was reported using 10–20 µg/ml chloramphenicol to partially inhibit protein synthesis (3); 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 (4-6). 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_{600} 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 lipopolysaccharides (LPSs), an essential component of the outer membrane of gram-negative cells such as *Escherichia coli* and *Salmonella typhimurium*, are present at about 3.5×10^6 copies/cell (7). 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 copurification of endotoxin. Other purification procedures such as CsCl banding and standard alkaline lysis can also result in the copurification of endotoxins. Endotoxin contamination in plasmid isolates can cause cytotoxicity in transfection and microinjection procedures (8).

In contrast to other commercial plasmid purification media that use a silica matrix, the Mobius™ 200 Columns are packed with a highly hydrophilic ion exchange bead allowing plasmid DNA to be selectively purified without copurification 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 (9).

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 preparing 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 with bacterial cultures; therefore, it is best to avoid using autoclaved glassware and solutions.

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USA and Canada
Tel (800) 526-7319
novatech@novagen.com

Germany
Tel 0800 100 3496
techservice@merckbiosciences.de

United Kingdom and Ireland
Freefone 0800 622935
Toll Free 1800 409445
customer.service@
merckbiosciences.co.uk

All Other Countries
Contact Your Local Distributor
www.novagen.com
novatech@novagen.com

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