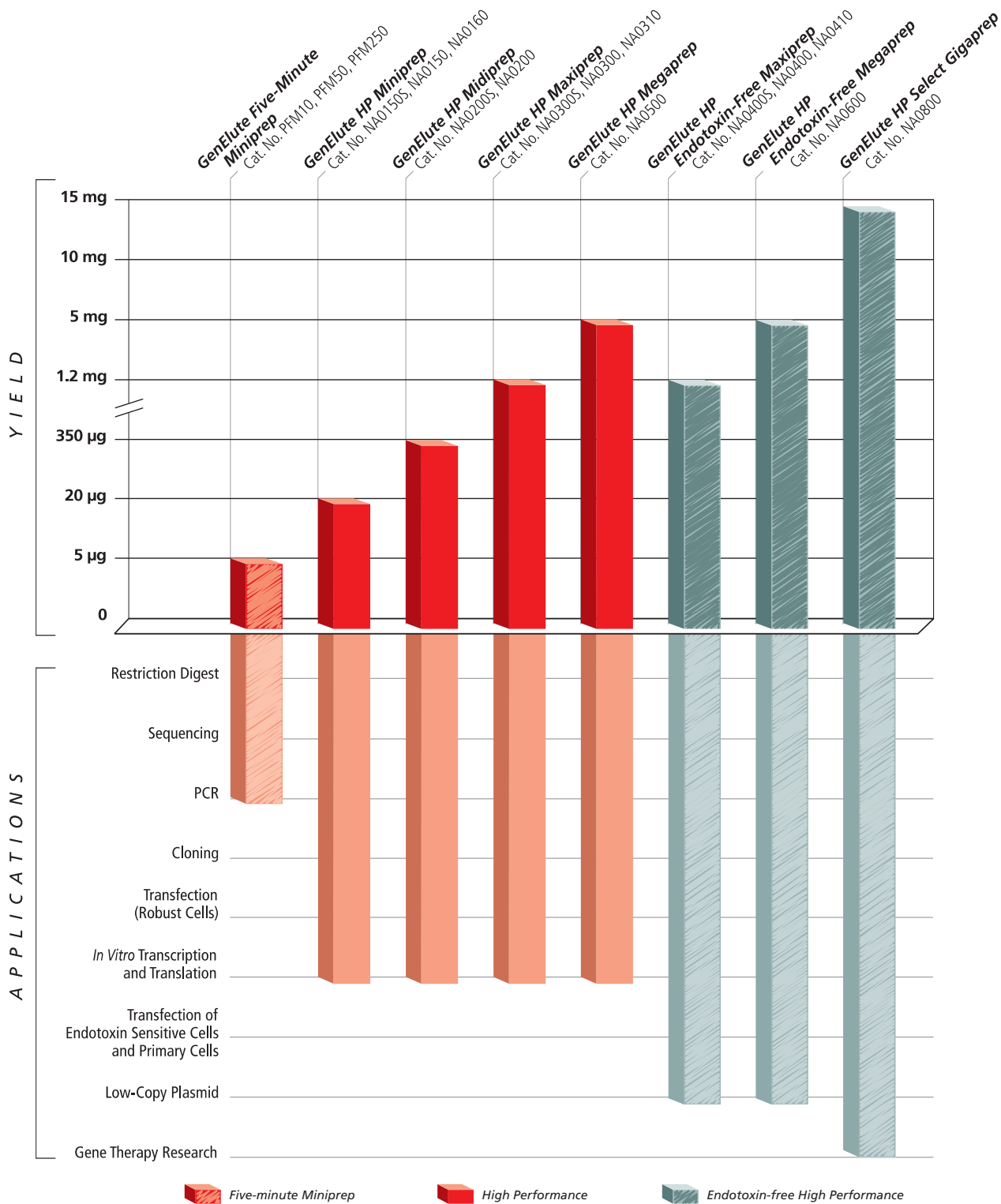


# HP Plasmid DNA Purification

## Sigma's GenElute™ DNA Plasmid Purification Kits

A Kit for Every Application — DNA Never Had It So Good!



# Plasmid DNA Purification

## GenElute™ Five-Minute Plasmid Miniprep Kit

The GenElute Five-Minute Miniprep Kit features an ultra streamlined protocol yielding up to 5 µg high-quality plasmid DNA in about five minutes.

**Procedure:** 400 µl of culture is added to a 2 ml collection tube. Cells are lysed for 1–2 minutes. A Binding Solution is added and the mixture is passed through a binding column. After washing the column to remove trace contaminants, the plasmid DNA is eluted in 10 mM Tris HCl, pH 8.5. Molecular biology water may be substituted as an elution reagent.

**Application:** The DNA is suitable for immediate use in capillary DNA sequencing, clone screening, restriction digestion and PCR.

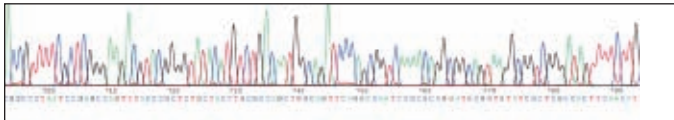
### Features and Benefits

- Sophisticated – no more pelleting cells or clearing lysates
- Flexible – binding column works with any standard laboratory vacuum manifold
- Plentiful – up to 5 µg of plasmid DNA or enough for 15 sequencing reactions
- Fast – only 5 minutes from start to finish
- Use only 400 µl overnight bacterial culture
- Choose vacuum or spin protocols

**Storage:** Room Temperature

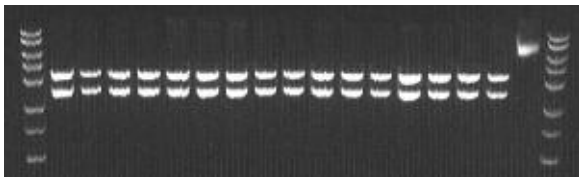
R: 10-36/38-67 S: 23-26-36/37

### Purified Plasmid DNA is Especially Suited for Automated Sequencing



**Figure 1. Capillary DNA sequencing of plasmid DNA purified with the GenElute Five-Minute Plasmid Miniprep Kit.** pCMV-SPORT-βgal (7.9 kb) was prepared from 400 µL of overnight culture of *E. coli* host strain DH5α™ with the GenElute Five-Minute Plasmid Miniprep Kit. A 5 µL sample of the preparation was submitted, with adjustment in DNA concentration, to SeqWright DNA Technology Service (Houston, TX) for sequencing with a T7 sequencing primer and ABI BigDye® Terminator v3.1 on an ABI Prism® 3730 DNA Analyzer. The Phred >20 score was 905 bases.

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19

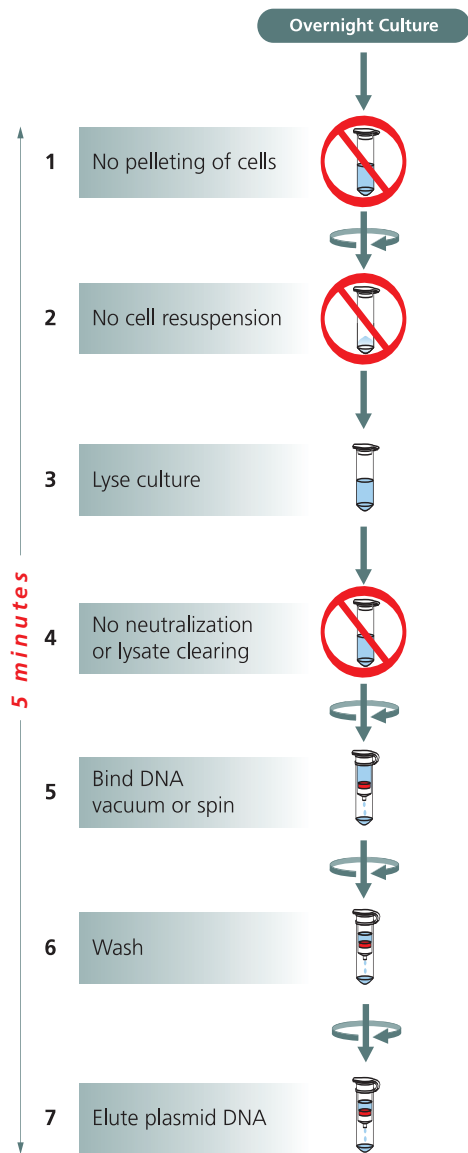


### Achieve Consistent Yields and Quality for Common Applications

**Figure 2. Restriction digestion analysis of pCMV-SPORT-βgal DNA purified with GenElute Five-Minute Plasmid Miniprep Kit.** Lanes 1 & 19: 1 kb DNA ladder (Cat. No. D0428); lanes 2 to 17: 16 independent plasmid mini preparations; lane 18: uncut pCMV-SPORT-βgal DNA. Each digestion was carried out in 15 µl at 37 °C for 1 hour, with 3 µl of eluate and 3 units each of *EcoR* I and *Xho* I. A quarter of each digestion was fractionated in 1% agarose gel.

### Ordering Information

Cat. No.	Product Description	Preps	Quantity
PFM10	GenElute™ Five-Minute Plasmid Miniprep Kit	10	1 kit
PFM50	GenElute™ Five-Minute Plasmid Miniprep Kit	50	1 kit
PFM250	GenElute™ Five-Minute Plasmid Miniprep Kit	250	1 kit



# Plasmid DNA Purification

## GenElute™ Plasmid Miniprep Kit

*For isolation of plasmid DNA from E. coli cultures*

The GenElute Plasmid Miniprep Kits offer simple, rapid, and cost-effective methods for isolating plasmid DNA from *E. coli* cultures. These kits combine silica-based membrane technology and the convenience of a spin column format. These kits also recover up to 20 µg of high copy plasmid DNA per ml of overnight culture.

Bacterial cells are harvested via centrifugation, subjected to a modified alkaline-SDS lysis procedure and the DNA adsorbed onto silica in the presence of high salts. Contaminants are then removed by a simple wash step.

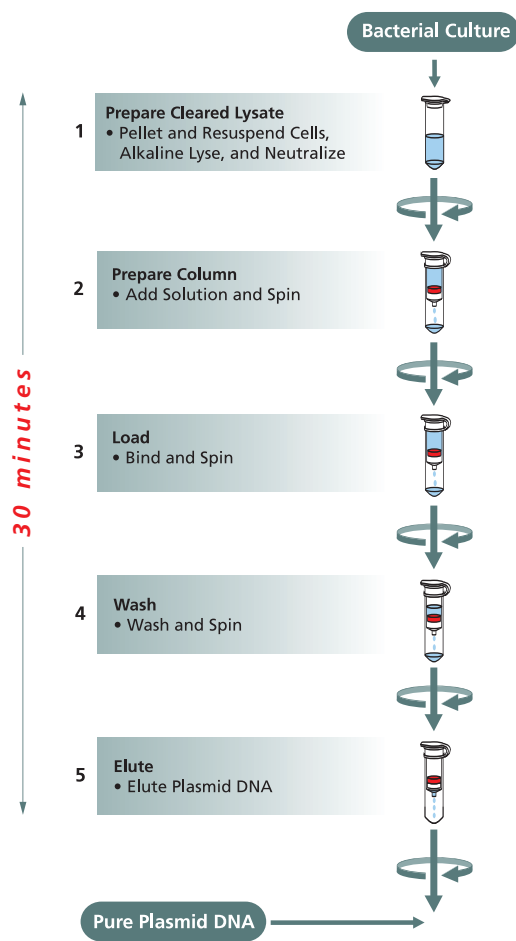
Finally, the bound DNA is eluted in water or Tris-EDTA buffer. The recovered plasmid DNA is predominately in its supercoiled form. There is no visual evidence of genomic DNA or RNA contamination. The DNA is ready for immediate use in applications such as restriction enzyme digestion, cloning, PCR, transformation, transcription, conventional and automated sequencing.

### Features and Benefits

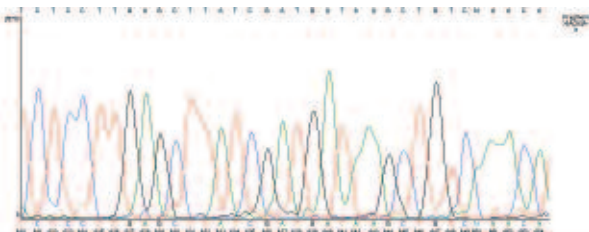
- 40% more preps per kit than the leading supplier
- Purify up to 20 µg of plasmid DNA per ml of culture
- Purified plasmid DNA in less than 30 minutes for up to 24 preps
- Faster than gravity flow anion exchange columns
- No detectable genomic DNA or RNA contamination
- No phenol/chloroform extraction or alcohol precipitation required
- Contains additional wash buffer for use with *EndA*<sup>+</sup> *E. coli* bacterial strains (e.g., HB101 JM101, BL21)

**Storage:** Room Temperature

R: 10-21/22-36/37/38 S: 16-26-36/37



### High Quality Plasmid DNA for Automated Sequencing



Electropherogram revealing >600 bases of sequence purified from pACD4 using GenElute™ Plasmid Miniprep Kit. The product was sequenced directly using BigDye® terminator v 3.1 chemistry. Sequencing reactions were analyzed on an ABI 3730xl.

### Ordering Information

Cat. No.	Product Description	Preps	Quantity
PLN10	GenElute™ Plasmid Miniprep Kit	10	1 kit
PLN70	GenElute™ Plasmid Miniprep Kit	70	1 kit
PLN350	GenElute™ Plasmid Miniprep Kit	350	1 kit

# Plasmid DNA Purification

## GenElute™ HP Mini, Midi, and Maxiprep Plasmid Kits

*Plasmid DNA from a maxiprep in less than 30 minutes*

The GenElute HP Plasmid Purification kits yield high quality plasmid DNA in less than 30 minutes (Fig. 1). GenElute HP Plasmid kits feature a filter syringe for lysate clearing, and binding columns that can be used with a vacuum or spin purification format.

An overnight recombinant *E. coli* culture is harvested by centrifugation and subjected to a modified alkaline-SDS lysis procedure. The lysate is cleared with a filter syringe and DNA is bound to the silica-based membrane. The remaining contaminants are removed by a wash step. Finally, the bound DNA is eluted in buffer or water (Fig. 2). High quality DNA is suitable for the most demanding applications including transfection (Fig. 3), restriction digestion (Fig. 4), ligation, automated sequencing (Fig. 5).

### Features and Benefits

- From harvested bacterial culture to pure plasmid DNA in 30 minutes or less
- Up to 25 µg (Mini), 350 µg (Midi), and 1.2 mg (Maxi) yield of high-copy plasmid DNA
- Offers the flexibility of a vacuum or spin format
- Contain fewer plastic components than other high speed kits, reducing the amount of waste
- No phenol/chloroform extraction or alcohol precipitation required
- Kits are stable at room temperature for convenient storage
- Cost effective

**Storage:** Room Temperature  
R: 22-36/37/38 S: 26-36

*"I spend less time purifying DNA and I get higher yields with the Sigma GenElute™ HP Plasmid Purification Kits. The kits give me time back that I can spend on other experiments."*

– Rene Rijnbrank, The University of Texas Medical Branch, Galveston



GenElute HP Plasmid Kits

### Preparation time for different maxiprep plasmid purification kits

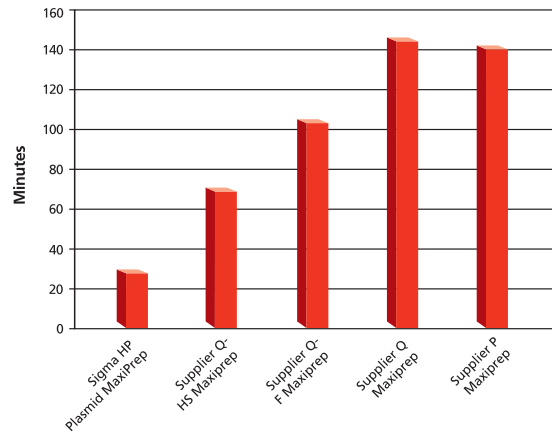


Figure 1. Comparison of time required per isolation using different Maxiprep plasmid isolation systems. Plasmid DNA was isolated following each manufacturer's recommended protocol. Each system was tested multiple times and the average preparation time is shown here.

### Plasmid DNA yield for different maxiprep kits

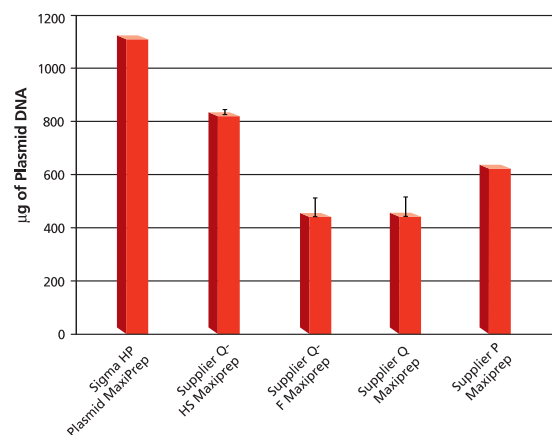
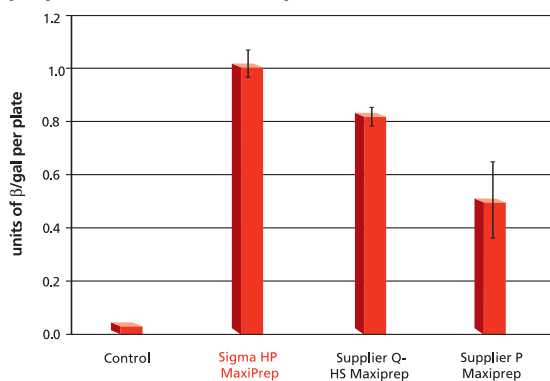


Figure 2. Comparison of plasmid DNA yield using different Maxiprep plasmid isolation systems. Plasmid DNA was isolated following each manufacturer's recommended protocol. All samples were done in duplicate.

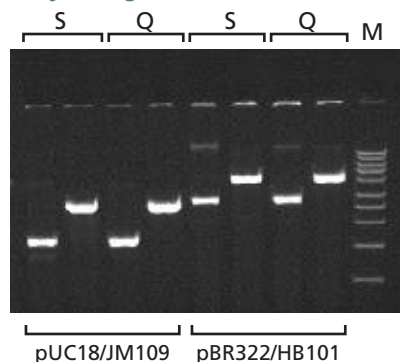
# Plasmid DNA Purification

## Comparison of transfection efficiencies of plasmids prepared with different plasmid isolation methods



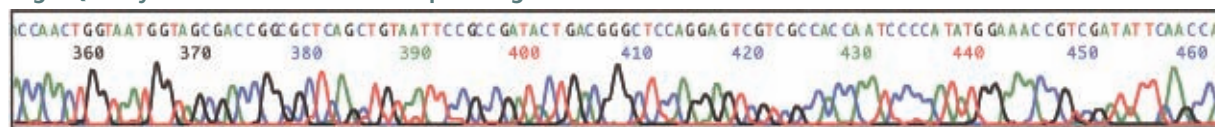
**Figure 3.** Transfection of cells with plasmid isolated using different plasmid purification kits. Data shows an average of 3 replicates for each named isolation method. All transfections were in CHO-K1 cells. The OD measurements were taken at 420 nm recording the units of β-gal/plate. The cells were grown to 60-70% confluency and transfected using 3 μg of plasmid pSPORT-CMV/15 μg of ESCORT™ IV. β-Gal activity was determined 54 hours post-transfection.

## Purified plasmid DNA is suitable for restriction enzyme digestions



**Figure 4.** Restriction digestion of plasmid DNA isolated using the GenElute™ HP Plasmid MaxiPrep Kit and a comparable kit from Supplier Q. The plasmid DNA (800 ng) was digested with 10 units of *Hind* III at 37 °C for 4 hours. Each plasmid sample represents 100 ng of undigested DNA (left) and digested DNA (right) loaded in each pair of lanes. Samples were loaded on a 1% TBE agarose gel. The marker (M) used was a 1 kb DNA Ladder (Cat. No. D0428).

## High Quality DNA for Automated Sequencing



**Figure 5.** Electropherogram revealing >700 bases of sequence from pCMV-SPORT-βgal purified from GenElute™ HP Plasmid MidiPrep Kit. Cycle Sequencing was performed using 500 ng template, a T7 sequencing primer and ABI BigDye® terminator chemistry. Sequencing reactions were resolved on an ABI Prism® 377 XL instrument with a 48 cm gel cassette containing AutoPAGE™ Plus 4.5% acrylamide at 2.4 kV, 48 °C for 7 hours. (Cat. No. P8977)

*The elution step can be modified for higher DNA concentration. After the addition of elution buffer, spin the column at 1000 x g for 5 minutes rather than the 3000 x g stated in the standard protocol. This will result in a reduced volume of eluate coming off the column which is approximately 3x more concentrated. This method delivers approximately 90% of the yield of the standard protocol.*

## Ordering Information

Cat. No.	Product Description	Preps	Quantity
NA0150S	GenElute™ HP Plasmid Miniprep Kit	10	1 kit
NA0150	GenElute™ HP Plasmid Miniprep Kit	70	1 kit
NA0160	GenElute™ HP Plasmid Miniprep Kit	350	1 kit
NA0200S	GenElute™ HP Plasmid MidiPrep Kit	4	1 kit
NA0200	GenElute™ HP Plasmid MidiPrep Kit	25	1 kit
NA0300S	GenElute™ HP Plasmid MaxiPrep Kit	4	1 kit
NA0300	GenElute™ HP Plasmid MaxiPrep Kit	10	1 kit
NA0310	GenElute™ HP Plasmid MaxiPrep Kit	25	1 kit

# Plasmid DNA Purification

## GenElute™ HP Plasmid Megaprep Kit

The GenElute HP Plasmid Megaprep Kit offers a simple, rapid, and cost-effective method for isolating plasmid DNA from recombinant *E. coli* cultures. This high performance kit features vacuum filter units for rapid lysate clearing and for DNA-silica binding. Yields of 5 mg of high copy plasmid DNA can be recovered from 200 ml-1.0 liter LB medium or 200-600 ml TB medium of *E. coli* culture in less than 1.5 hours.

**Procedure:** An overnight recombinant *E. coli* culture is harvested with centrifugation and subjected to a modified alkaline-SDS lysis procedure followed by adsorption of the DNA onto a silica membrane in the presence of high salts. Contaminants are removed by a vacuum wash step. The bound DNA is then eluted in Elution Solution (Tris-HCl) or water.

**Application:** High-quality DNA is ready for immediate use for the most demanding applications, including transfection, cloning, restriction digestion, automated sequencing, and PCR.

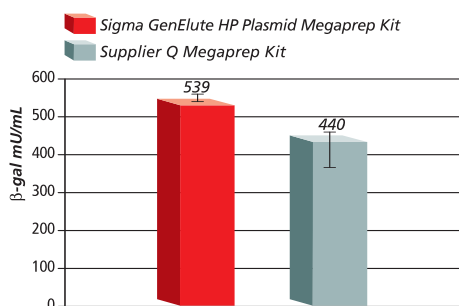
### Features and Benefits

- Sophisticated – newest technology ensures improved performance
- Plentiful – yields 5 mgs of high-quality, endotoxin-free ( $\leq 0.1\text{EU}/\mu\text{g}$ ) plasmid DNA in 90 minutes or less
- Convenient – vacuum format with no ethanol precipitation required

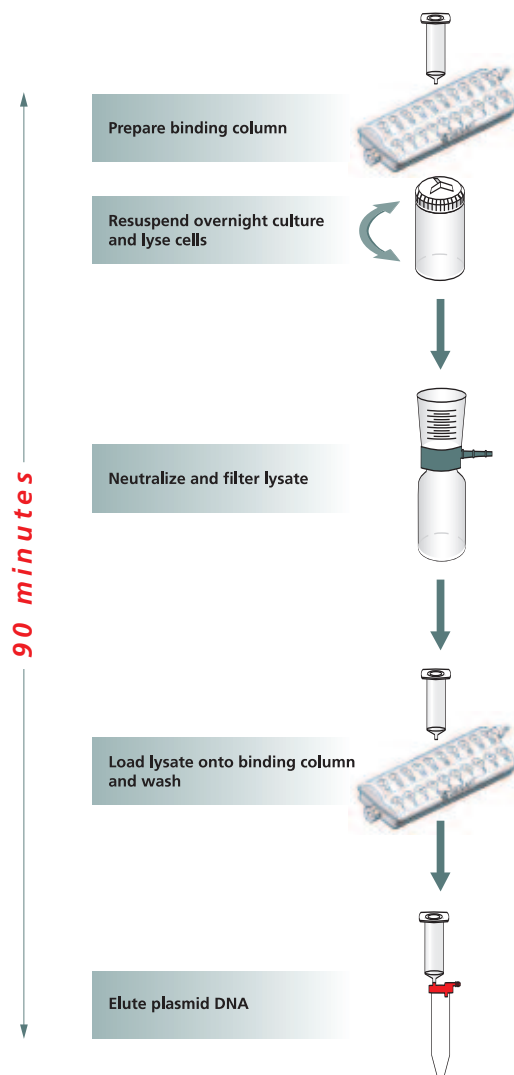
**Storage:** Room Temperature

R: 10-22-36/37/38-48/20-67 S: 53-26-36/37/39-45

### Transfection Efficiencies in CHOK1 Cells

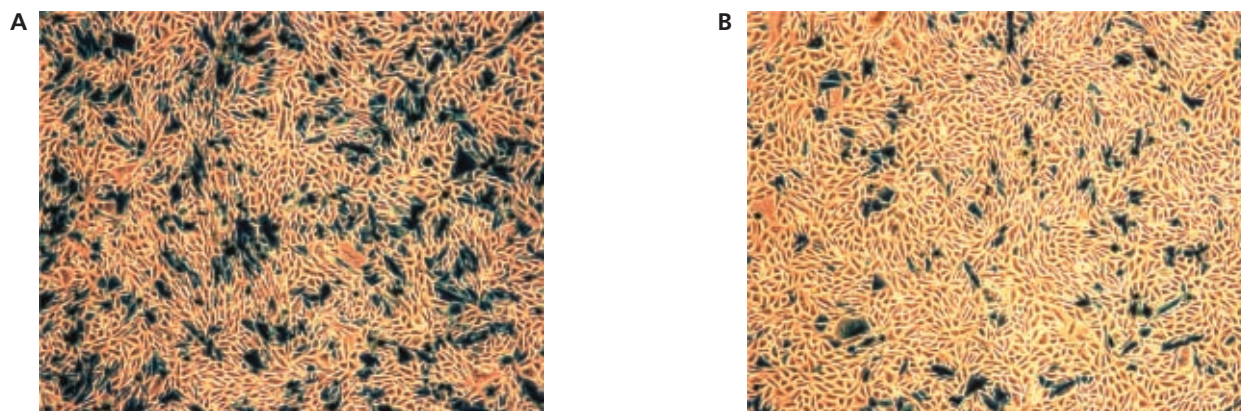


**Figure 1. Comparison of transfection efficiencies into CHOK1 cells using different purification systems with pCMV-SPORT- $\beta$ gal.** The data shows the average and standard deviations of three replicates for both kits evaluated. Untransfected CHOK1 cells reported no  $\beta$ -galactosidase activity (data not shown).



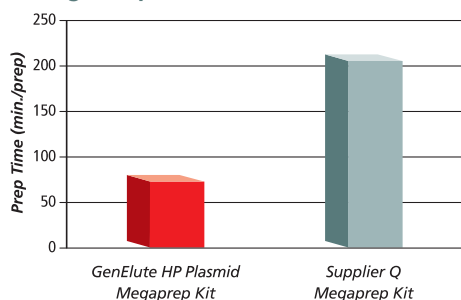
# Plasmid DNA Purification

## Comparison of transfection efficiencies of different purification systems using X-gal staining

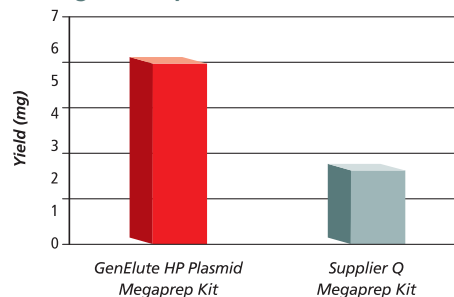


**Figure 2.** Panel A represents cells transfected with plasmid DNA isolated from the GenElute HP Plasmid Megaprep Kit; Panel B represents cells transfected with plasmid DNA isolated with Supplier Q's Megaprep Kit. CHOK-1 cells were transfected with 3  $\mu$ g of plasmid DNA using ESCORT™ II transfection reagent, using plasmid DNA isolated from a GenElute HP Plasmid Megaprep Kit, and a kit from Supplier Q. The panels above show  $\beta$ -gal staining 48 hours post-transfection. Higher transfection efficiency is clearly evident in Panel A, with the plasmid DNA isolated with the GenElute HP Kit.

### Average Prep Time



### Average Yield per Time



**Figure 3.** Comparison of plasmid yield and time/prep for the different purification systems used for isolating plasmid pCMV1-Sport- $\beta$ gal. The table on the left depicts the average time per preparation of the Sigma GenElute HP Plasmid Megaprep Kit as opposed to Supplier Q's Megaprep Kit. The figure on the right depicts the average yield obtained by the GenElute HP Plasmid Megaprep as compared to the Supplier Q Megaprep Kit.

*To ensure the optimal yield, prepare a seed culture by inoculating 5 ml of liquid media (containing the appropriate antibiotic) with a single colony from a freshly streaked plate and incubating for a few hours. Then use the seed culture to inoculate the larger culture.*

*[Note: We do not recommend inoculating liquid cultures directly from a glycerol stock. Cells from the glycerol stock that do not contain plasmid can occasionally survive and replicate after antibiotic has been sufficiently depleted, reducing the yield.]*

## Ordering Information

Cat. No.	Product Description	Preps	Quantity
NA0500	GenElute™ HP Plasmid Megaprep Kit	5	1 kit

# Plasmid DNA Purification

## GenElute™ Endotoxin-free Plasmid Midiprep Kit

*Purify endotoxin-free plasmid DNA for high efficiency transfection*

Endotoxins (also known as lipopolysaccharides or LPS) are often co-purified with plasmid DNA and can significantly reduce transfection efficiencies in sensitive cell lines. The GenElute Endotoxin-free Plasmid Midiprep Kit offers a simple, rapid, cost-effective method for purifying plasmid DNA with  $\leq 0.1$  EU/ $\mu\text{g}$  DNA for high efficiency transfection (Figs. 1 and 2).

Recombinant *E. coli* is harvested from an overnight culture by centrifugation and subjected to a modified alkaline-SDS lysis procedure to produce a cleared lysate. Endotoxins are removed from the cleared lysate with simple extraction-phase separation steps. Plasmid DNA is further purified by adsorption onto silica in the presence of high salts. After a simple spin-wash step, the bound plasmid DNA is eluted in endotoxin-free water. Up to 250  $\mu\text{g}$  of endotoxin-free plasmid DNA may be prepared from 5 to 40 ml of culture.

The recovered plasmid DNA is predominately in its super-coiled form. There is no visual evidence of genomic DNA or RNA contamination on ethidium bromide-stained agarose gels. The DNA is ready for immediate use in downstream applications such as transfection, restriction endonuclease digestion, cloning, sequencing, and PCR amplification.

### Features and Benefits

- Higher transfection efficiency and yield than market leaders
- Purify up to 250  $\mu\text{g}$  endotoxin-free plasmid DNA ( $\leq 0.1$  EU/ $\mu\text{g}$  DNA)
- Fast and simple, allowing up to 12 preps in less than 2 hours
- Faster than gravity flow ion exchange columns or magnetic bead preps
- No expensive magnetic equipment required

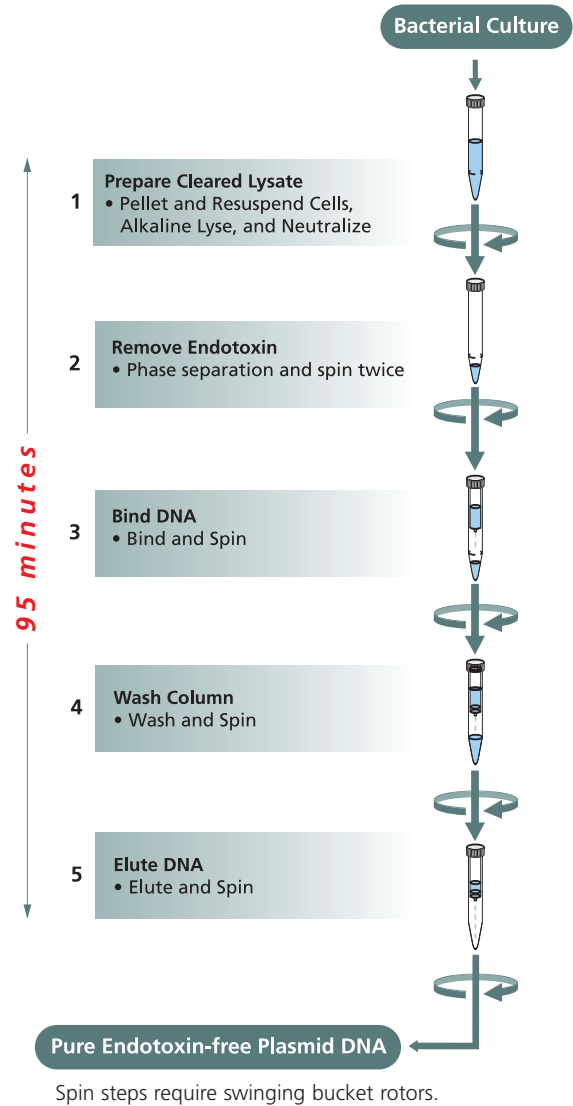
**Storage:** Room Temperature

R: 34-20/21/22 S: 26-27-36/37/39-45

*For optimal yield, use fresh overnight cultures when purifying plasmid DNA with our kits. If cultures must be stored, harvest the cells by centrifugation and decant the liquid media, then store the pellets at  $-70$  °C for up to one month. If a  $-70$  freezer is not available, the pellets can be stored at  $-20$  °C for 2-3 days without adversely affecting plasmid recovery.*

### Ordering Information

Cat. No.	Product Description	Preps/Kit	Culture Volume	Expected Yield
PLED35	GenElute™ Endotoxin-free Plasmid Midiprep Kit	35	5-40 ml	Up to 250 $\mu\text{g}$





# Plasmid DNA Purification

## GenElute™ HP Endotoxin-free Plasmid Maxiprep Kit

GenElute HP Endotoxin-free Plasmid Maxiprep Kit offers a novel and efficient method of isolating endotoxin-free plasmid DNA in 40 minutes. A patent-pending endotoxin removal process has resulted in the fastest, most streamlined protocol available for purifying high-quality, endotoxin-free DNA ( $\leq 0.1 \text{ EU}/\mu\text{g}$ ).

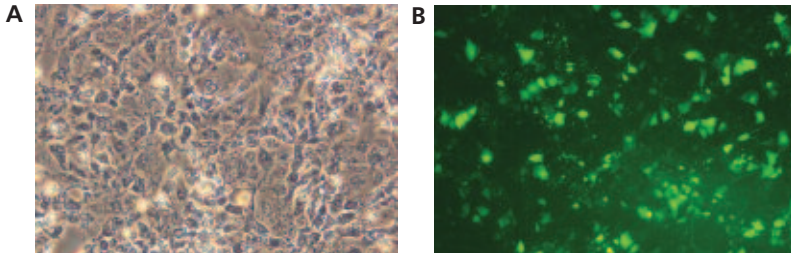
### Features and Benefits

- Fast – from harvested bacterial culture to pure plasmid DNA in 40 minutes
- Flexible – convenient vacuum format does not use gravity flow columns
- High Yields – up to 1.2 mg of high-copy plasmid DNA with  $\leq 0.1 \text{ EU}/\mu\text{g}$
- Economic – lower cost + higher yields = significant savings

**Storage:** Room Temperature  
 R: 22-36/37/38-67 S: 7-23-26-36

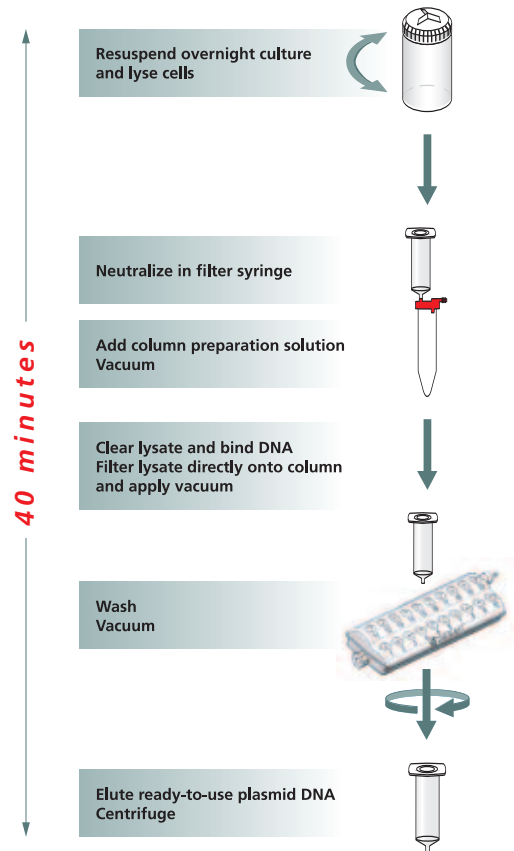
Cells Under Bright Field

Cells Under Fluorescent Exposure



**Assessment of transfection efficiency using plasmid pCop-Green-C.** Plasmid pCop-Green-C is a mammalian expression vector that encodes the copepod green fluorescent protein. HuH-7 cells were seeded onto glass cover slips and then grown to 60-70% confluency. The cells were then transfected with 3  $\mu\text{g}$  of plasmid DNA using ESCORT™ II transfection reagent. Plasmid DNA was isolated from a GenElute HP Endotoxin-free Maxiprep. At 72 hours post transfection, the cells were observed under a fluorescence microscope. **Panel A** is a picture of the cells following transfection under bright field exposure. **Panel B** is the same area of cells under fluorescent exposure, which reveals a high transfection efficiency.

\* pCop-Green-C is licensed from Evrogen, JSC.



### High Yields and Low Endotoxin Levels in Less Time

Purification System	Plasmid Yield (mg)	Endotoxin Levels (EU/ $\mu\text{g}$ )	Time/Prep (minutes)
GenElute HP Endotoxin-free Maxiprep Kit	1.4	0.02	35
Endo-free Anion-Exchange-based Kit	0.7	0.04	165
Endo-free Silica-Magnetic-based Kit	1.3	0.17	150
2x Cesium Chloride-gradient	0.3	1.4	3 days

Comparison of plasmid yield, endotoxin levels and time/rep for the different purification systems for pCMV-SPORT- $\beta$ -gal.

### Ordering Information

Cat. No.	Product Description	Preps	Quantity
NA04005	GenElute™ HP Endotoxin-free Plasmid Maxiprep Kit	4	1 kit
NA0400	GenElute™ HP Endotoxin-free Plasmid Maxiprep Kit	10	1 kit
NA0410	GenElute™ HP Endotoxin-free Plasmid Maxiprep Kit	25	1 kit

# Plasmid DNA Purification

## GenElute™ HP Endotoxin-free Plasmid Megaprep Kit

Isolate endotoxin-free plasmid DNA in 90 minutes or less with the GenElute HP Endotoxin-free Plasmid Megaprep Kit. The kit features a patent-pending endotoxin removal system for isolation of DNA with  $\leq 0.1 \text{ EU}/\mu\text{g}$ . With a convenient vacuum format and no need for alcohol precipitation, this kit provides the fastest method for isolation of endotoxin-free plasmid DNA.

**Procedure:** An overnight recombinant *E. coli* culture is harvested by centrifugation and subjected to a modified alkaline-SDS lysis. The lysate is cleared by filtration followed by the addition of a binding solution that has been optimized for endotoxin-free plasmid preparations. The plasmid DNA is then captured onto a silica membrane in the presence of high salts, while endotoxins are prevented from adsorbing to the membrane. Contaminants are removed by two wash steps. The bound DNA is then eluted in endotoxin-free water.

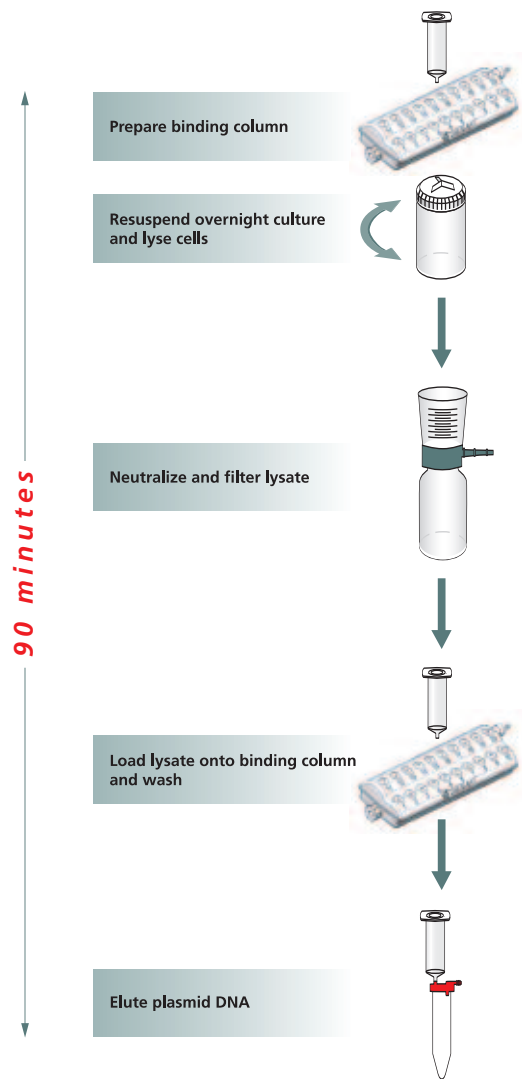
**Application:** High-quality, endotoxin-free DNA is ready for immediate use for the most demanding applications including transfection with endotoxin-sensitive cells

### Features and Benefits

- Fast – from harvested bacterial culture to pure plasmid DNA in 90 minutes
- Convenient – vacuum format with no ethanol precipitation required
- High Yields – 5 mg of high-copy plasmid DNA ( $\leq 0.1 \text{ EU}/\mu\text{g}$ )
- Economic – lower cost, higher yields and significantly reduced labor equals substantial savings — in both dollars and time

**Storage:** Room Temperature

R: 10-22-36/37/38-48/20-67 S: 26-36/37/39-45



### Plentiful Yield for Downstream Applications

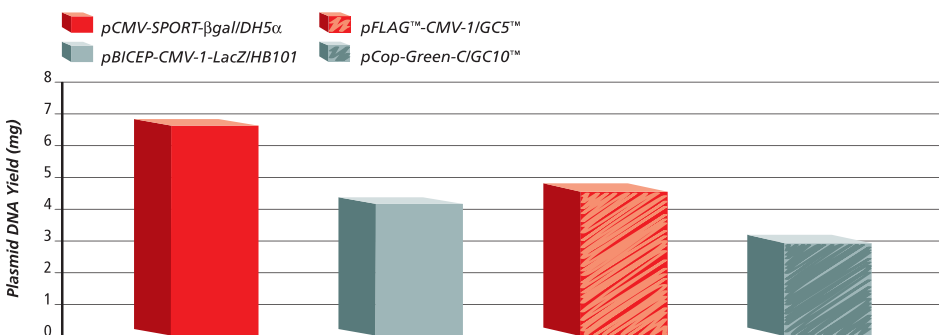


Figure 1. Maximum plasmid DNA yield obtained with different constructs using GenElute HP Endotoxin-free Plasmid Megaprep Kit.

# Plasmid DNA Purification

## High Yields and Low Endotoxin Levels in Less Time

Purification Kit	Plasmid Yield (mg)	Endotoxin Levels (EU/μg)	Time/Prep (minutes)
GenElute HP Endotoxin-free Megaprep Kit	6.0	1-10	75
Supplier Q Endotoxin-Free Plasmid Mega Kit	2.5	1-45	210
Supplier B Plasmid Mega EF Kit	2.0	39-46	210
Supplier M High Purity Plasmid Megaprep System	1.7	13-38	90

Comparison of plasmid yield, endotoxin levels and time/prep for the different purification systems used for isolating high copy number plasmid pCMV-SPORT-β-gal/DH5α™ in LB.

## Transfect Endotoxin-Sensitive Cell Lines

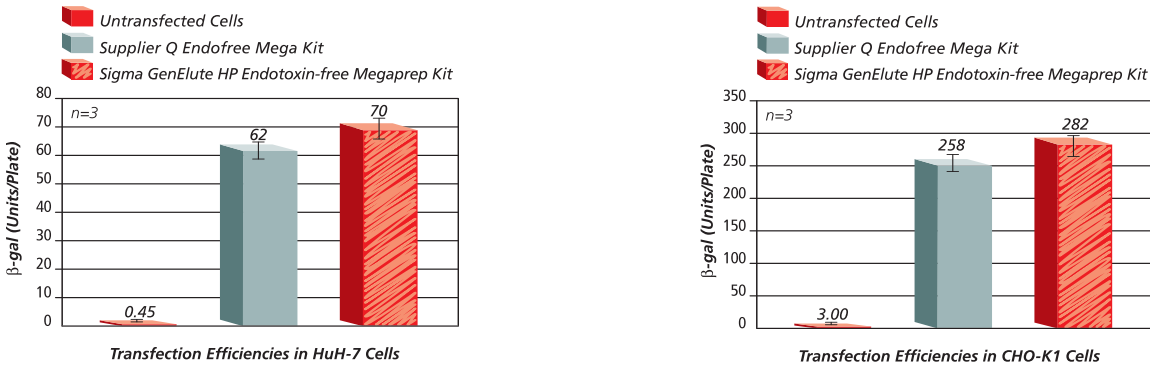


Figure 2. Comparison of transfection efficiencies of high-copy plasmid DNA pCMV-SPORT-β-gal/DH5α™ into endotoxin sensitive cell line HuH-7 cells and non-endotoxin sensitive cell line CHO-K1 cells. The data shows the average and standard deviations of three replicates for both cell lines evaluated. Untransfected HuH-7 and CHO-K1 cells represent cells treated with transfection reagents without any plasmid DNA.

*"I switched to Sigma's GenElute™ HP Plasmid Purification Kits because the yield is much higher, the purity is better or equal to my previous method and, importantly, it takes me only a quarter of the time."*

– Waldemar Popik, Johns Hopkins University

## Ordering Information

Cat. No.	Product Description	Preps	Quantity
NA0600	GenElute™ HP Endotoxin-free Plasmid Megaprep Kit	5	1 kit

# Plasmid DNA Purification

## GenElute™ HP Select Plasmid Gigaprep Kit

The GenElute HP Select Plasmid Gigaprep Kit offers a simple, rapid, and cost-effective method for isolating endotoxin-free plasmid DNA from recombinant *E. coli* cultures. Yields of 15 mg of high copy plasmid DNA with  $\leq 0.1$  EU/ $\mu$ g can be recovered from *E. coli* cultures. High-quality, endotoxin-free DNA can be used for the most demanding applications including transfection with endotoxin-sensitive cells and gene therapy research.

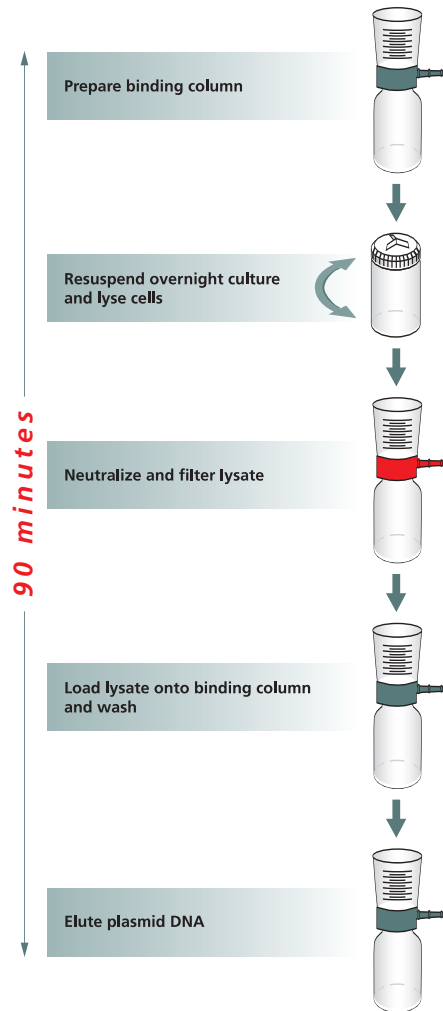
**Procedure:** An overnight recombinant *E. coli* culture is harvested by centrifugation and subjected to a modified alkaline-SDS lysis. The lysate is cleared by filtration followed by the addition of a binding solution optimized for endotoxin-free plasmid preparations. The plasmid DNA is then captured onto a silica membrane in the presence of high salts, while endotoxins are prevented from adsorbing to the membrane. Contaminants are removed with three wash steps. Finally, the bound DNA is eluted in endotoxin-free water.

**Application:** High-quality, endotoxin-free DNA is ready for immediate use for the most demanding applications including transfection with endotoxin-sensitive cells and gene therapy research.

### Features and Benefits

- Sophisticated – utilizes HP Select technology, the premier plasmid purification method
- Novel – includes patent-pending endotoxin removal system
- High Yields – 15 mg of high-quality, endotoxin-free ( $\leq 0.1$  EU/ $\mu$ g) plasmid DNA in 2 hours or less
- Convenient – vacuum format with no ethanol precipitation required
- Versatile – can be used to purify low-, medium-, and high-copy plasmid DNA

**Storage:** Room Temperature  
R: 10-22-36/37/38-48/20-67 S: 53-26-36/37/39



### More Plasmid DNA in Significantly Less Time

Purification System	Plasmid Yield (mg)	Time/Prep (hours)	Alcohol Precipitation Required?
GenElute HP Select Plasmid Gigaprep Kit	12.2	1.5	NO
Supplier Q Endo-Free Plasmid Giga Kit	10.9	4.8	YES
Supplier B Plasmid Giga EF Kit	6.3	3.3	YES

Comparison of plasmid yield and time/prep for the different purification systems used for isolating medium-copy number plasmid pFLAG-CMV1/DH5 $\alpha$  in LB medium.

# Plasmid DNA Purification

## Isolate DNA from Low-, Medium-, and High-copy Plasmids

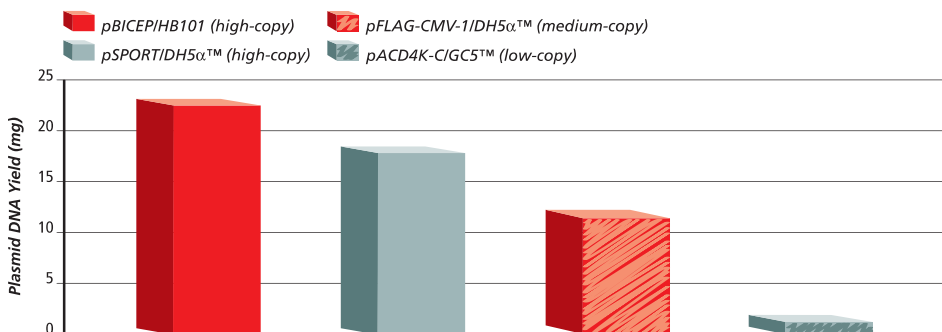


Figure 1. Maximum plasmid DNA yield obtained with different constructs using GenElute HP Select Plasmid Gigaprep Kit.

## Transfect Endotoxin-Sensitive Cell Lines

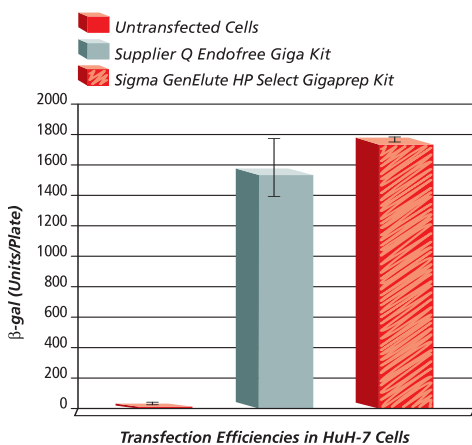


Figure 2. Comparison of transfection efficiencies of high copy plasmid DNA pCMV-SPORT-β-gal/DH5α™ into endotoxin sensitive cell line HuH-7 cells. The data shows the average and standard deviations of three replicates for the cell line evaluated. Untransfected HuH-7 represent cells treated with transfection reagents without any plasmid DNA.

*The lysate clearing agent functions as a prefilter, eliminating the need for an extra step when preparing cleared lysates. Just add the clearing agent to the lysate, mix thoroughly, wait five minutes, and filter.*

## Ordering Information

Cat. No.	Product Description	Preps	Quantity
NA0800	GenElute™ HP Select Plasmid Gigaprep Kit	5	1 kit

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## PhasePrep™ BAC DNA Kit

*A highly scalable and rapid method for isolating BAC DNA*

The PhasePrep BAC DNA Kit offers a highly scalable, rapid, cost-effective method for isolating high molecular weight plasmids such as Bacterial Artificial Chromosomes (BACs) from recombinant *E. coli* cultures. The kit utilizes a phase separation procedure that can be scaled to micro, mini, midi, or maxi prep sizes. Sufficient reagents are provided for 300 micro, 180 mini, 30 midi, or 15 maxi preps. Up to 2, 12, 60, or 100 µg of BAC DNA can be recovered from 5, 40, 250, or 500 ml of overnight recombinant *E. coli* culture, respectively. The purified BAC DNA contains very low levels of endotoxins and is ready for immediate use in automated sequencing (Fig. 1), PCR, restriction enzyme digestion (Fig. 2), cloning and other common applications.

### Features and Benefits

- Typical DNA yields of 2-100 µg from 5-500 ml of overnight cultures
- No phenol/chloroform extraction required
- Allows possible micro to maxi preps with the same kit
- No long waits for drip columns

**Storage:** Room Temperature

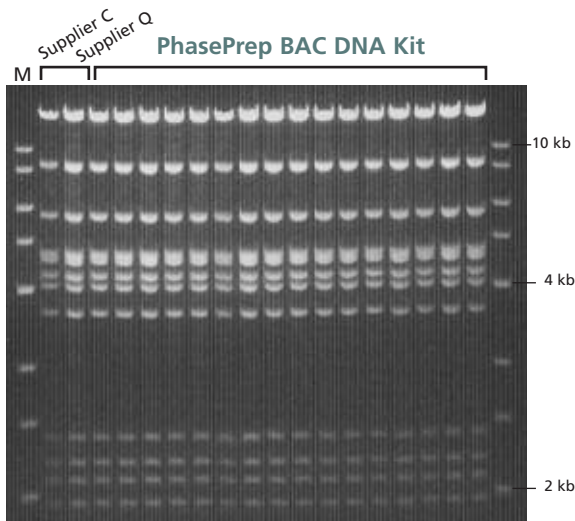
R: 11-34-25-67 S: 16-26-37/39-45

### High Quality BAC DNA for Automated Sequencing



Figure 1. BAC DNA was purified with the PhasePrep™ BAC DNA Kit and sequenced with a custom primer using BigDye® Terminator Chemistry. Sequencing reactions were resolved on an ABI 3700.

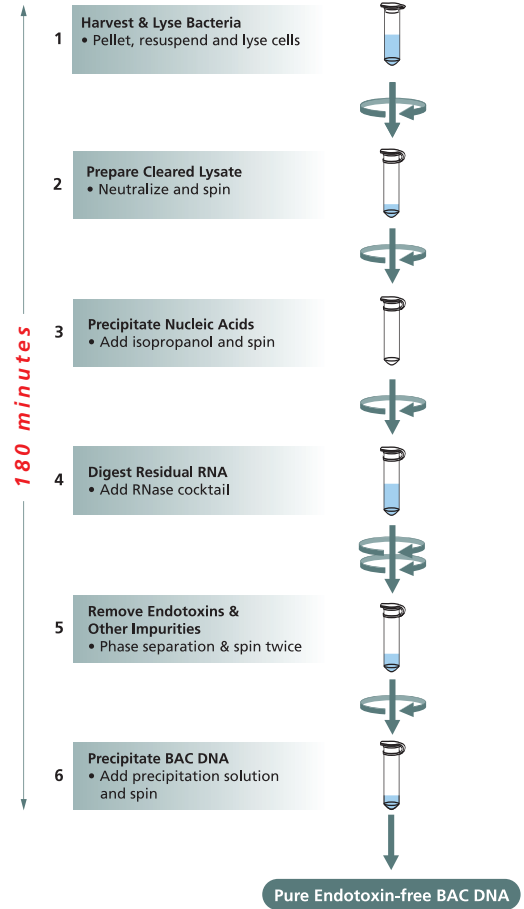
Data provided by the Genome Sequencing Center at Washington University, St. Louis



### Purified BAC DNA is suitable for restriction digestion.

Figure 2. Restriction digestion of BAC DNA isolated with the PhasePrep™ BAC DNA Kit and comparable kits from Supplier C and Supplier Q. BAC DNA samples were purified from overnight cultures of *E. coli* HB101b transformed with a pbelloBAC11 clone. Approximately 1 µg of DNA from each sample was digested with 20 units of *EcoR* V at 37 °C for 4 hr. One half of each digestion (approximately 0.5 µg) was separated by overnight electrophoresis in 1% agarose gel. Lanes 4-19 were BAC DNA purified with Sigma Kit, lane 1 was BAC DNA purified with supplier C's kit, and lane 2 was BAC DNA purified with Supplier Q's kit. The molecular marker used at the first and last lane was the 1 kb DNA Ladder (Cat. No. D0428).

### Bacterial Culture



### Ordering Information

Cat. No.	Product Description	Culture Volume	Expected Yield
NA0100	PhasePrep™ BAC DNA Kit	5-500 ml	2-100 µg