

GE Healthcare



illustra
plasmidPrep
Midi Flow kit

For extraction and purification of transfection quality
plasmid DNA from *E. coli*

Product booklet

Codes: 28-9042-67 (25 purifications)
28-9042-68 (100 purifications)



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Quick Reference Protocol Card

Back Cover

Tear off sheet containing a protocol for the experienced user preparing high-copy or low-copy number plasmids.

1. Legal

Product use restriction

The components of the **illustra plasmidPrep Midi Flow Kit** have been designed, developed and sold for research purposes only. They are suitable for *in vitro* use only. No claim or representation is intended for its use to identify any specific organism or for clinical use (diagnostic, prognostic, therapeutic, or blood banking).

It is the responsibility of the user to verify the use of the **illustra plasmidPrep Midi Flow Kit** for a specific application as the performance characteristics of this kit have not been verified for any specific organism.

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GE Healthcare UK Limited.

Amersham Place, Little Chalfont,
Buckinghamshire. HP7 9NA UK.

2. Handling

2.1. Safety warnings and precautions

Warning: For research use only.

Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.

All chemicals should be considered as potentially hazardous. Only persons trained in laboratory techniques and familiar with the principles of good laboratory practice should handle these products. Suitable protective clothing such as laboratory overalls, safety glasses and gloves should be worn. Care should be taken to avoid contact with skin or eyes; if contact should occur, wash immediately with water (see Material Safety Data Sheet(s) and/or Safety Statements(s) for specific recommendations).

Warning: This protocol requires the use of Ethanol.

Lysis buffer type 11 contains Ribonuclease; sensitizer. Lysis buffer type 12 contains Sodium Hydroxide; irritant. Lysis buffer type 13 contains Acetic Acid; irritant. Wear gloves and safety glasses.

2.2. Storage







This kit is shipped at ambient temperature and can be stored at room temperature (20–25°C). Ensure temperature of Lysis buffers type 11 & 13 is reduced to 4°C prior to use. For convenience, Lysis buffers type 11 & 13 can be stored at 4°C.





2.3. Expiry

For expiry date please refer to outer packaging label.

3. Components

3.1. Kit contents

Identification	Pack Size	5	25	100
		purifications	purifications	purifications
	Cat. No.	Sample pack	28-9042-67	28-9042-68
	Lysis buffer type 11 (Red colored cap)	36 ml	180 ml	2 × 360 ml
	Lysis buffer type 12 (Clear colored cap)	36 ml	180 ml	2 × 360 ml
	Lysis buffer type 13 (Blue colored cap)	36 ml	180 ml	2 × 360 ml
	Wash buffer type 2 (Yellow colored cap)	120 ml	2 × 300 ml	5 × 480 ml
	Elution buffer type 3 (Silver colored cap)	24 ml	120 ml	480 ml
	TE buffer type 1 (Green colored cap)	6 ml	30 ml	120 ml

Identification	Pack Size	5	25	100
		purifications purifications purifications		
	Cat. No.	Sample pack	28-9042-67	28-9042-68
	illustra™ Fast-Flow plasmid 250 columns*	5	25	2 × 50
	Column stand*	Not included*	1	1
	Waste reservoir*	Not included*	1	1
	Adapters†	6	12	12

Refer to the Certificate of Analysis for a complete list of kit components.

*The cardboard package supplied with 25 and 100 pack sizes can be converted into a column stand to aid purification. The plastic column tray can be used as a Waste reservoir.

†The Adapters assist the use of racks and centrifuge tubes with the illustra Fast-Flow plasmid 250 columns during the Purification step, allowing elution off the Fast-Flow column directly into a high speed centrifuge tube.

GE Healthcare supplies a wide range of buffer types across the illustra nucleic acid purification and amplification range. The composition of each buffer has been optimized for each application and may vary between kits. Care must be taken to only use the buffers supplied in the particular kit you are using and not use the buffers supplied in other illustra kits e.g. the Lysis buffers supplied in the illustra plasmidPrep Midi Flow Kit are not the same as the Lysis buffers supplied in the illustra plasmidPrep Mini Spin Kit.

In order to avoid confusion and the accidental switching of buffers between kits, a numbering system has been adopted that relates to

the entire range of buffers available in the illustra purification range. For example there are currently 14 Lysis buffers in the illustra range, 6 Wash buffers and 8 Elution buffers, denoted by Lysis buffer type 1-14, Wash buffer type 1-6 and Elution buffer type 1-8, respectively. Please ensure you use the correct type of Lysis, Wash and Elution buffer for your purification.

3.2. Materials and reagents to be supplied by user

Disposables:

1.5 ml DNase-free microcentrifuge tubes (1 per purification)

Ice

Chemicals:

70% Ethanol

Isopropyl Alcohol (Propan-2-ol)

Sterile media and antibiotics for culture of bacteria (see sections 5.2 & 6.4 for further details)

3.3. Equipment to be supplied by user

Standard microbiological equipment and consumables, including culture plates, loops, flasks and 37°C shaking incubator.

Tube cutter (preferably 4 mm), guillotine or scissors.

LabMate™ PD-10 Buffer Reservoirs can be used for easier handling and loading of volumes greater than 12 ml onto the illustra Fast-Flow plasmid 250 column.

Centrifuge(s) and tubes:

Please ensure you have a suitable centrifuge, rotor and tube types for the centrifugation steps detailed in Table 1 below. Tubes must be able to withstand the spin speeds indicated. In addition, for the centrifugation within the Concentration and De-salination steps, the centrifuge tubes must be able to withstand alcohol. All centrifugation steps are performed at 4°C.

Table 1: Centrifugation details

Plasmid copy number	Details	Bacterial Culture Preparation step	Lysis & Clarification steps	Purification step (very low copy number only)	Concentration step	De-salination step
High (low)	Sample volume (ml)	50 (150)	18		8	5
	Spin speed (× g)	5 000	20 000		15 000	15 000
	Length of spin (minutes)	15	30		30	10
	Tube composition		polypropylene or polycarbonate		polypropylene or polyallomer	polypropylene or polyallomer
Very low	Sample volume (ml)	500	45	90	8	5
	Spin speed (× g)	5 000	20 000	20 000	15 000	15 000
	Length of spin (minutes)	15	2 × 20	30	30	10
	Tube composition		polypropylene or polycarbonate	polypropylene or polyallomer	polypropylene or polyallomer	polypropylene or polyallomer

The different tube types are represented pictorially in the protocols as follows:

Polypropylene or polycarbonate (use for Lysis & Clarification steps)



Polypropylene or polyallomer (use for Purification, Concentration and De-salination steps)



4. Description

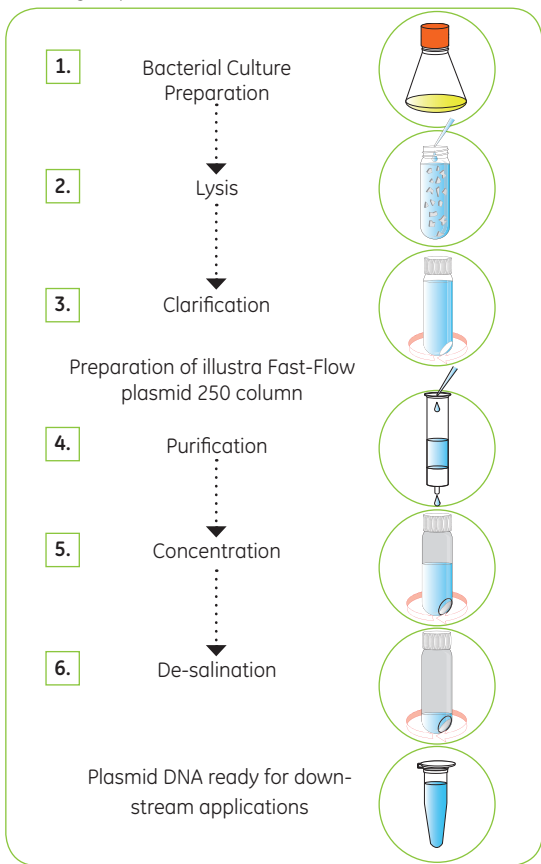
4.1. Introduction




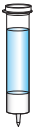


The **illustra plasmidPrep Midi Flow Kit** is designed for the extraction and purification of transfection quality plasmid DNA from *Escherichia coli* (*E. coli*). The protocol is based on a simple process, at the core of which are the 4 steps of Lysis, Clarification, Purification and De-salination. A modified alkaline Lysis procedure is followed by clarification through centrifugation, to remove precipitated genomic DNA, Potassium Dodecyl Sulphate (KDS) and other contaminants. Anion exchange Purification is facilitated by plasmid binding in high-salt conditions to the illustra Fast-Flow plasmid 250 columns. Impurities such as RNA, cell wall debris and protein are removed through successive high-salt washes. Purified plasmid is eluted from the column at a higher salt concentration. The plasmid is then concentrated and de-salinated using Isopropanol precipitation and a 70% Ethanol wash.


illustra Fast-Flow plasmid 250 columns are provided pre-equilibrated and require only gravity to run, reducing hands on and process time. The resin bed of the illustra Fast-Flow plasmid 250 columns is protected with a high density polyethylene Vyonfilter frit that prevents the Fast-Flow plasmid 250 media from running dry during the Purification step. The column medium has been developed to provide excellent flow characteristics, exceptional chemical and pH stability, and high selectivity and capacity for plasmid DNA. The large effective surface area and high level of derivitization of the medium facilitate strong anionic-exchange. Since plasmid DNA binds at a high salt concentration, binding of potential impurities is minimized. Pure plasmid can be eluted in buffers containing higher salt concentrations.

4.2. The basic principle

Use of the **illustra plasmidPrep Midi Flow Kit** involves the following steps:



Step	Comments	Component
1. Bacterial Culture Preparation	<i>E. coli</i> is grown to late logarithmic phase in appropriate medium and harvested by centrifugation.	Bacteria
2. Lysis	Bacterial pellet is re-suspended in buffer containing RNase I. Cells are lysed in alkaline conditions with Sodium Dodecyl Sulphate (SDS)-detergent present. Genomic DNA and proteins are denatured; RNA is hydrolyzed. Neutralization with a buffer containing Potassium Acetate causes genomic DNA, protein and KDS to precipitate.	Lysis buffer type 11  Lysis buffer type 12  Lysis buffer type 13 
3. Clarification	High speed centrifugation causes genomic DNA, protein and KDS precipitate to form a pellet. Plasmid DNA remains in solution.	
Preparation of illustra Fast-Flow plasmid 250 column		illustra Fast-Flow plasmid 250 column 
4. Purification	Clarified lysate is loaded onto illustra Fast-Flow plasmid 250 column. Plasmid binds to the anion exchange matrix. Wash steps remove contaminants and pure plasmid is eluted in a high-salt buffer.	Wash buffer type 2  Elution buffer type 3 

Step	Comments	Component
5. Concentration	Plasmid is concentrated by Isopropanol precipitation and recovered by high speed centrifugation.	
6. De-salination	Salt and Isopropanol are removed with a 70% Ethanol wash. Plasmid DNA is recovered by high speed centrifugation. The pellet is air dried and re-suspended in a low ionic strength buffer.	TE buffer type 1 

4.3. Product specifications

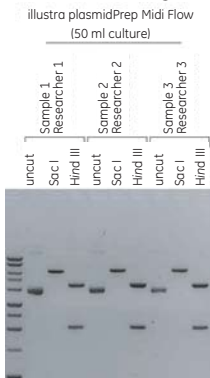
Sample type:	<i>E. coli</i> cultures containing high, low or very low-copy number plasmids and cosmid vectors up to 50 kbp
Lysis principle	Modified alkaline lysis procedure
Purification principle	Anion-exchange chromatography
Purification format	Single-use, gravity flow illustra Fast-Flow plasmid 250 columns
Effective capacity	≥ 250 µg plasmid DNA
Sample size	From 25 ml up to 150 ml culture medium, dependent upon growth conditions, strain of <i>E. coli</i> and size and copy number of vector (see section 5.2 & 6.4 for details)
Typical yield	100 to ≥ 250 µg high-copy number plasmid 20 to 250 µg low and very low-copy number plasmid

Sample type:	<i>E. coli</i> cultures containing high, low or very low-copy number plasmids and cosmid vectors up to 50 kb.
Purity	
A_{260}/A_{280}	1.8–2.0
A_{260}/A_{230}	2.0–2.5
% supercoiled DNA	> 70% (strain dependent)
Major subsequent applications	Transfection of most cell lines, automated sequencing, PCR and mutagenesis, enzyme modifications and cloning.

If large numbers of samples are to be processed from small volumes of bacterial cultures (1–3 ml), transfection quality DNA is not required and a yield of 6–9 µg plasmid DNA per purification is sufficient, consider use of the illustra plasmidPrep Mini Spin Kit.

4.4. Typical output

Figure 1. Purification of a 6.3 kb high copy number plasmid from *E. coli* TOP10 using the **illustra plasmidPrep Midi Flow Kit**



The agarose gel shows that uncut samples consist predominantly of supercoiled monomeric plasmid DNA (pCORON1002-EGFP-C1). No endogenous nuclease activity was evident. Complete restriction enzyme digestion was effected with SacI and HindIII. 3 separate 50 ml culture volumes were processed by 3 separate researchers.

Table 2a: Typical yield & purity values

Plasmid details	Culture Volume (ml)	Yield (ug)	A ₂₆₀ /A ₂₈₀	A ₂₆₀ /A ₂₃₀
High copy number, 6.3 kbp	50	289	1.90	2.34
Low copy number, 36 kbp	150	191	1.93	2.29

Table 2b: Typical quality values

Plasmid details	Culture Volume (ml)	Endotoxin (EU/mg)	Supercoiled monomer (%)	Transfection (% COS7 cells)	Sequencing (Phred 20 read length)
High copy number, 6.3 kbp	50	0.05	91.5	72	818
Low copy number, 36 kbp	150	0.13	ND	ND	ND

High copy number and low copy number plasmids were prepared using the illustra plasmidPrep Midi Flow Kit, as indicated. ND = not determined.

5. Protocols



Note: It is advisable to familiarize yourself with Sections 3.2, 3.3 and 5 before beginning the purification, ensuring all material, reagents, consumables and equipment are to hand.



Note: Ensure appropriate tubes are available for high speed centrifugation steps



Note: Buffers are NOT transferable between GE Healthcare illustra kits e.g. the composition of the Wash buffer in the plasmidPrep Midi Flow Kit is not the same as the Wash buffer in the plasmidPrep Mini Spin Kit. Please note type number for differentiation.

Use of icons

The Key below describes the purpose of the icons used throughout the protocol booklet.



This icon is used to highlight particularly critical steps within the protocol that must be adhered to. If this advice is not followed it will have a detrimental impact on results.



This icon is used to highlight technical tips that will enhance the description of the step. These tips may indicate areas of flexibility in the protocol or give a recommendation to obtain optimum performance of the kit.




The protocol can be stopped at a number of convenient points that are denoted by the stop sign.



Purification analysis: To assess the efficiency of key steps in the purification process it may be pertinent, especially when using the kit for the first time, to remove aliquots (where you see





the arrow sign) during the Purification step for subsequent UV spectrophotometry and agarose gel analysis. See section 6.3 for processing and analysis of these samples.

 **Note:** Two protocols are provided; one for the purification of up to 250 µg of high and low copy number plasmid and one for purification of very low-copy number plasmids (< 10 copies per cell). Please ensure you follow the correct protocol (see Table 3).

5.1. Preparation of working solutions

See section 3.2 and 3.3 for Materials & Equipment to be supplied by user.

Lysis buffers type 11 & 13

Ensure temperature of **Lysis buffer type 11**  and **Lysis buffer type 13**  is reduced to 4°C before use.

Lysis buffer type 12

Ensure no precipitate is visible in the bottle containing **Lysis buffer type 12**. If necessary, warm the buffer in a 37°C water bath for 5 minutes. **Lysis buffer type 12** should be stored at room temperature (20–25°C).

5.2. Essential preliminaries

For a more in depth analysis of the factors affecting plasmid yield, purity and quality, please see section 6.4.

Culture volumes


 Culture volumes of *E. coli* required differ depending on the plasmid copy number (see Table 3). The volumes advised enable optimal performance of the Fast-Flow plasmid 250 columns. To obtain good aeration, use a sterile flask with a volume of at least 4 times the volume of the culture.

Table 3: Relationship between plasmid copy number, culture volume, expected yield and protocol

Plasmid copy number	Maximum culture volume (ml)	Expected yield (μg)	Protocol
High (> 50)	25–50	100–250	Section 5.3
Low (10–50)	150	20–250	Section 5.3
Very low (< 10)	500	20–200	Section 5.4

*If cell density exceeds 1×10^{10} cells/ml harvest only 25 ml of culture.

***E. coli* strain**

The protocols provided have been optimized using a range of strains of *E. coli*. Certain strains of *E. coli* produce excessive carbohydrate, especially when combined with the larger culture volumes required for very low copy number plasmid purification. When purifying very low copy number plasmids, the protocol in section 5.4 should be followed, where the volume of **Lysis buffers type 11, 12 & 13** used is increased to 10 ml during the Lysis step. In addition 3 washes with 10 ml **Wash buffer type 2** is performed during the Purification step. Additional buffers can be made according to the recipes provided in section 6.6.

5.3. Protocol for purification of high-copy number and low-copy number plasmids

1. Bacterial Culture Preparation

- a. Pick a single colony from a freshly streaked selective plate and inoculate a starter culture of 2–5 ml Lysogeny Broth (LB) medium containing the appropriate selective antibiotic. (LB can also be known as Luria broth or Luria Bertani broth). Adjust salt levels as appropriate for bacterial strain, culture conditions and salt sensitivity of antibiotic used. Incubate for approximately 8 hours, 37°C, 225 rpm.



Starter culture
2–5 ml
selective LB
8 hours 37°C
225 rpm



Note: To obtain good aeration, use a sterile tube or flask with a volume of at least 4 times the volume of the culture.

- b. Dilute the starter culture 1 in 1000 into the appropriate volume of selective LB medium (see Table 4 below). Incubate at 37°C 12–18 hours, 225 rpm.



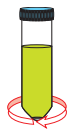
Dilute 1:1000
into 50–150 ml
selective LB
12–18 hours 37°C
225 rpm

Table 4: Typical growth criteria with DH5 α and TOP10 cells

Plasmid copy number	Maximum culture volume (ml)	Inoculum volume (μ l)	Expected final cell density	Typical final A_{600}
High (> 50)	50*	50	$3-9 \times 10^9$ *	1.3–2.6
Low (10–50)	150	150	$3-9 \times 10^9$	1.3–2.6

*If cell density exceeds 1×10^{10} cells/ml harvest only 25 ml of culture.


- c. To harvest cells from the medium, centrifuge for 15 minutes at $5\,000 \times g$ and 4°C . Pour off spent culture medium and use a pipet to remove any excess fluid and leave the cell pellet as dry as possible.

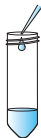


15 minutes
 $5\,000 \times g$
 4°C

STOP The cell pellet may be stored at -20°C . Defrost thoroughly at room temperature for 5–15 minutes prior to use. Do not thaw for greater than 15 minutes, as endogenous enzyme activity can affect plasmid yield and purity.

2. Lysis

- a. Cell resuspension-Add 6 ml **Lysis buffer type 11**  and thoroughly re-suspend the pellet by pipetting up and down.




6 ml Lysis buffer
type 11

- b. Transfer to high speed centrifuge tube.

⚠ Note: Ensure tubes selected are made of polycarbonate or polypropylene, suitable for centrifugation at $20\,000 \times g$ and large enough to allow complete mixing of **Lysis buffers types 11–13** added in the following steps.




- c. Cell lysis-Add 6 ml **Lysis buffer type 12** . Mix thoroughly by inverting 6 times with an incisive action. Do not vortex, as this will result in shearing of genomic DNA.




6 ml Lysis buffer
type 12

- d. Incubate at room temperature (RT) for 5 minutes. The lysate will appear viscous.


5 minutes
RT

 **Note:** Do not incubate for longer than 5 minutes as this may denature supercoiled plasmid DNA.

- e. Neutralization-Add 6 ml pre-chilled **Lysis buffer type 13**  and immediately mix thoroughly by inverting with an incisive action 6 times.



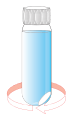
6 ml Lysis buffer
type 13

 **Note:** A white flocculent will form and viscosity should be reduced. If the mixture still appears viscous, more mixing is required. Do not vortex, as this will result in shearing of genomic DNA.

- f. Incubate on ice for 15 minutes.


3. Clarification

- a. Spin the sample for 30 minutes at 20 000 × g and 4°C.



15 minutes
on ice

30 minutes
20 000 × g
4°C

 **Note:** Use polypropylene or polycarbonate tubes.

Preparation of Fast-Flow plasmid 250 column


- a. During the centrifugation, remove the top cap from the Fast-Flow plasmid 250 column. Cut off the recessed part of the bottom tip using a guillotine, 4 mm tube-cutters or scissors. Allow the excess storage liquid to flow through the column. Support the column over a suitable waste receptacle, such as the Waste reservoir provided, to catch buffer flowthrough.




4. Purification

- a. Column loading-Immediately following centrifugation, carefully pipet the clarified lysate supernatant into a new polycarbonate or polypropylene tube.





 **Note:** After centrifugation the supernatant should be clear. If supernatant is not clear, transfer to fresh tube and re-centrifuge. Immediate transfer of the clarified lysate with a pipet to a fresh tube before loading onto column is recommended to avoid disturbing the pellet. The small flecks of flocculent that remain on the surface of the clarified lysate should stick to the pipet.

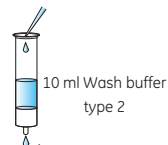
 Purification analysis: Remove 50 μ l of clarified lysate supernatant.


- b. Apply 10 ml of clarified lysate directly to the Fast-Flow plasmid 250 column. Apply remaining clarified lysate to column. Allow lysate to enter the resin completely by gravity flow.



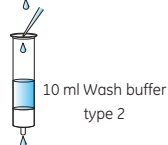
 Purification analysis: Retain 100 μ l of flowthrough.


- c. Wash-Apply 10 ml **Wash buffer type 2**  to column. Allow buffer to completely enter the resin by gravity flow.




 Purification analysis: Retain 200 μ l of flowthrough.


- d. Repeat wash with additional 10 ml **Wash buffer type 2** .




 **Note:** This second wash step is essential for high purity.

← Purification analysis: Retain 200 μ l of flowthrough.

e. Elute-Place a suitable polyallomer or polypropylene centrifuge tube under the column. Use the Adapters to ensure flowthrough enters tube. Apply 4 ml **Elution buffer type 3**  to the column and collect the flowthrough.


 **Note:** Use of polycarbonate centrifuge tubes is not recommended as they are not resistant to the alcohol used in subsequent steps. Use polypropylene or polyallomer tubes.

← Purification analysis: Retain 40 μ l flowthrough.

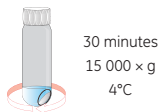
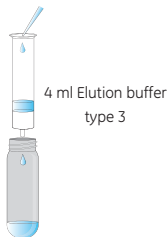
 Eluate may be stored at 4°C overnight or at -20°C for longer periods.


5. Concentration

a. Add 4 ml room temperature Isopropanol. Mix by inversion.

 **Note:** Ensure Isopropanol is at room temperature to minimize salt precipitation.

b. Mark the outside of the tube to aid pellet location and immediately spin for 30 minutes at 15 000 \times g and 4°C. The tube should be placed in the rotor such that the mark is located where the pellet is expected to form.



 Ensure centrifugation is performed at 4°C to prevent sample overheating.

- c. Carefully and immediately pour off supernatant or aspirate with a pipet. The pellet will have a glassy appearance but can be located by means of the prior tube mark. Take care not to disturb the plasmid pellet when removing supernatant.



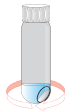
6. De-salination

- a. Wash the pellet with 5 ml room temperature 70% (v/v) Ethanol.



5 ml 70%
Ethanol


- b. Spin for 10 minutes at 15 000 × g and 4°C.




10 minutes
15 000 × g
4°C

- c. Carefully pour off the supernatant or aspirate with a pipet.




 **Note:** The pellet may be distributed over the wall of the tube, and so wash the walls and pellet with 70% Ethanol prior to centrifugation. Take care not to disturb the plasmid pellet when removing supernatant.

- d. Air dry the pellet for 5–10 minutes. Dissolve plasmid DNA in up to 1 ml **TE buffer type 1** . Transfer the sample to a 1.5 ml DNase-free microcentrifuge tube (user supplied).



≤ 1 ml TE buffer
type 1

 **Note:** Over drying the pellet will make the plasmid difficult to dissolve.

Rinse the walls of the tube to ensure recovery of all plasmid DNA.

Excessive pipetting may shear plasmid DNA.

If pellet was over dried, heat the TE buffer to 60°C prior to addition, or gently roller mix buffer on the pellet overnight.

- e. Determine plasmid yield, purity, endotoxin levels and quality as necessary (see section 6.2 for details).
- f. Store purified plasmid DNA at -20°C.

5.4. Protocol for purification of very low-copy number plasmids

1. Bacterial Culture Preparation

- a. Pick a single colony from a freshly streaked selective plate and inoculate a starter culture of 2–5 ml LB medium containing the appropriate selective antibiotic. Incubate for approximately 8 hours, 37°C, 225 rpm.



Starter culture
2–5 ml
selective LB
8 hours 37°C
225 rpm



Note: To obtain good aeration, use a sterile tube or flask with a volume of at least 4 times the volume of the culture.

- b. Dilute the starter culture 1 in 1000 into selective LB medium – inoculate 500 ml of LB medium with 500 µl of starter culture. Incubate at 37°C 12–18 hours, 225 rpm.

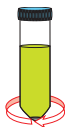


Dilute 1:1000 into
500 ml selective LB
12–18 hours
37°C 225 rpm



Note: Final cell density should be 3–9 × 10⁹/ml, with typical A₆₀₀ values of 1.3–2.6 for standard bacterial strains such as TOP10 and DH5α.


c. To harvest cells from the medium, spin for 15 minutes at $5\,000 \times g$ and 4°C . Pour off spent culture medium and use a pipet to remove any excess fluid and leave the cell pellet as dry as possible.



15 minutes
 $5\,000 \times g$
 4°C

STOP The cell pellet may be stored at -20°C . Defrost thoroughly at room temperature for 5–15 minutes prior to use. Do not thaw for greater than 15 minutes, as endogenous enzyme activity can affect plasmid yield and purity.

2. Lysis

a. Cell resuspension-Add 15 ml **Lysis buffer type 11**  and thoroughly re-suspend the pellet by pipetting up and down.



15 ml Lysis buffer
type 11

b. Transfer to high speed centrifuge tubes.

⚠ Note: Ensure tubes selected are made of polycarbonate or polypropylene, are suitable for centrifugation at $20\,000 \times g$ and are large enough to allow complete mixing of Lysis buffers types 11–13 added in the following steps.



c. Cell lysis-Add 15 ml **Lysis buffer type 12** . Mix thoroughly by inverting 6 times with an incisive action.




15 ml Lysis buffer
type 12

d. Incubate at room temperature (RT) for 5 minutes. The lysate will appear viscous.

5 minutes
(RT)

⚠ Note: Do not incubate for longer than 5 minutes as this may denature supercoiled plasmid DNA.

Do not vortex, as this will result in shearing of genomic DNA.


- e. Neutralization-Add 15 ml pre-chilled **Lysis buffer type 13**  and immediately mix thoroughly by inverting with an incisive action 6 times.



15 ml
Lysis buffer
type 13

- f. Incubate on ice for 15 minutes.

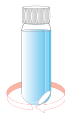
15 minutes
on ice

 **Note:** A white flocculent will form and viscosity should be reduced. If the mixture still appears viscous, more mixing is required.

Do not vortex, as this will result in shearing of genomic DNA.

3. Clarification

- a. Spin the sample for 20 minutes at $20\,000 \times g$ and 4°C .



20 minutes
 $20\,000 \times g$
 4°C

- b. Pour supernatant into a fresh polycarbonate or polypropylene high speed centrifuge tube.




- c. Re-centrifuge for 20 minutes at $20\,000 \times g$ and 4°C . After the second centrifugation, the supernatant should be clear.



20 minutes
 $20\,000 \times g$
 4°C

- d. Immediately pour or pipet supernatant into a fresh polycarbonate or polypropylene high speed centrifuge tube.



 **Note:** Immediate transfer of the clarified lysate with a pipet to a fresh tube before loading onto column is recommended to avoid disturbing the pellet. The small flecks of flocculent that remain on the surface of the clarified lysate should stick to a pipet.

Preparation of Fast-Flow plasmid 250 column

a. During the centrifugation, remove the top cap from the Fast-Flow plasmid 250 column. Cut off the recessed part of the bottom tip using a guillotine, 4 mm tube-cutters or scissors. Allow the excess liquid to flow through the column. Support the column over a suitable waste receptacle, such as the Waste reservoir provided, to catch buffer flow through.



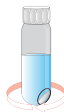
4. Purification

a. Add 45 ml Isopropanol to the clarified lysate and mix by inversion.




45 ml Isopropanol

b. Centrifuge for 30 minutes at 20 000 × g and 4°C.





30 minutes
20 000 × g
4°C

c. Carefully remove supernatant and dissolve pellet in 4 ml **TE buffer type 1**  by pipetting up and down.




4 ml TE buffer
type 1

 **Note:** Take care not to disturb the pellet when removing the supernatant.

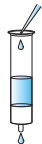
d. Add 6 ml **Wash buffer type 2** , mix by inversion.



6 ml Wash buffer
type 2


 Purification analysis: Retain 50 µl of clarified lysate supernatant.

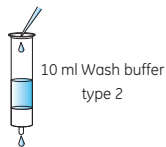
e. Apply sample directly to Fast-Flow plasmid 250 column and allow to enter media completely by gravity flow.



Apply clarified
lysate

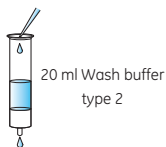
← Purification analysis: Retain 100 µl of flowthrough.


f. Wash-Apply 10 ml **Wash buffer type 2**  to column. Allow buffer to completely enter the resin by gravity flow.




← Purification analysis: Retain 200 µl of flowthrough.

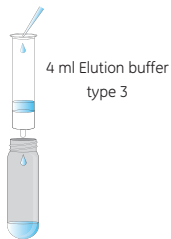
g. Repeat wash twice with additional 20 ml **Wash buffer type 2** .




 **Note:** These additional washes are essential when purifying very low copy number plasmids from large culture volumes or using bacterial strains that produce large amounts of carbohydrate. A total of 3×10 ml washes is required.


← Purification analysis: Retain 200 µl of flowthrough.

h. Elute-Place a suitable polyallomer or polypropylene centrifuge tube under the column. Use the Adapters to ensure flowthrough enters tube. Apply 4 ml **Elution buffer type 3**  to the column and collect the eluate.



 **Note:** Use of polycarbonate centrifuge tubes is not recommended as they are not resistant to the alcohol used in subsequent steps.

← Purification analysis: Retain 40 µl flowthrough.

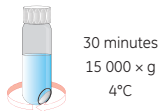
 Eluate may be stored at 4°C overnight or at -20°C for longer periods.


5. Concentration

a. Add 4 ml room temperature Isopropanol. Mix by inversion.



b. Immediately spin for 30 minutes at 15 000 \times g and 4°C. Carefully and immediately pour off supernatant or aspirate with a pipet.



 **Note:** Ensure Isopropanol is at room temperature to minimize salt precipitation.

Ensure centrifugation is performed at 4°C to prevent sample overheating.

The pellet will have a glassy appearance and may be difficult to locate. Mark the outside of the tube before centrifugation to aid pellet location.



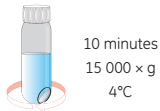
Take care not to disturb the plasmid pellet when removing supernatant.

6. De-salination

a. Wash the pellet with 5 ml room temperature 70 % (v/v) Ethanol.




b. Spin for 10 minutes at 15 000 \times g and 4°C.



c. Carefully pour off the supernatant or aspirate with a pipet.




 **Note:** The pellet may be distributed over the wall of the tube, and so wash the walls and pellet with 70% Ethanol prior to centrifugation. Take care not to disturb the plasmid pellet when removing supernatant.

d. Air dry the pellet for 5–10 minutes. Dissolve plasmid DNA in up to 1 ml **TE buffer type 1**



≤ 1 ml TE buffer
type 1

 **Note:** Over drying the pellet will make the plasmid difficult to dissolve.

Rinse the walls of the tube to ensure recovery of all plasmid DNA.

Excessive pipetting may shear plasmid DNA.

If pellet was over dried, heat the TE buffer to 60°C prior to addition, or gently roller mix buffer on the pellet overnight.

e. Determine plasmid yield, purity, endotoxin levels and quality as necessary (see section 6.2 for details).

f. Store purified plasmid DNA at -20°C.

6. Appendices

6.1. RPM calculation from RCF

The appropriate centrifugation speed for a specific rotor can be calculated from the following formula:

$$\text{RPM} = 1000 \times \sqrt{(\text{RCF}/1.12r)}$$

Where RCF = relative centrifugal force; r = radius in mm measured from the center of the spindle to the bottom of the rotor bucket; and RPM = revolutions per minute.

For example, if an RCF of $735 \times g$ is required using a rotor with a radius of 73 mm, the corresponding RPM would be 3 000.

6.2. Plasmid yield, purity, endotoxin level & quality determination

Yield

Purified plasmid concentration should be determined by UV spectrophotometry (A_{260}), and through comparison with a known standard by agarose gel electrophoresis and subsequent densitometric analysis. Typical yield values are given in table 5 below.

Table 5: Expected yield for different plasmid types

Plasmid copy number	Maximum culture volume (ml)	Expected yield (μg)
High (> 50)	50	100–250
Low (10–50)	150	20–250
Very low (< 10)	500	20–200



Note: An absorbance reading of 1 at 260 nm corresponds to approximately 50 ng/ μl .

The reliable UV spectrophotometric range should be determined for each spectrophotometer. Generally, for spectrophotometers with a 1 cm path length, A_{260} readings that lie between 0.1 and 1.0 can be trusted and therefore appropriate dilutions (5 to 50 ng/ml) should be analyzed. For Nano-Drop™ spectrophotometers, absorbance readings between 1 and 10 are reliable.

Purity

If available, the UV spectrophotometric ratios A_{260}/A_{280} and A_{260}/A_{230} provide a limited indication of purity as measures of protein, RNA and salt contamination.

Expected A_{260}/A_{280} range is 1.8–2.0, and A_{260}/A_{230} is 2.0–2.5. If there is significant contamination with protein, the A_{260}/A_{280} will be less, and accurate quantitation of plasmid DNA will not be possible by spectroscopy. Significant absorption at 230 nm can indicate salt contamination.

Endotoxin levels

Endotoxin levels can be determined by a number of methods such as the QCL-1000™ kit from Lonza Corp. Ltd.

Plasmid quality

Agarose gel electrophoresis and subsequent densitometric analysis can be used to determine the percentage of supercoiled DNA relative to genomic DNA and denatured supercoiled and nicked (linear and open circular) plasmid.

The majority of plasmid should be supercoiled when using the illustra plasmidPrep Midi Flow Kit. If your purified sample appears not to be predominantly supercoiled, please consult sections 6.4 & 6.5. See **Table 6** for guidelines of how to interpret agarose gel and spectroscopy results.

Table 6: Interpretation of agarose gel and spectroscopy results

Unexpected presence of	Agarose gel result	Spectroscopy shows
Protein	-	A_{260}/A_{280} ratio < 1.8
Salt	Plasmid fails to digest with restriction enzyme.	Significant absorption at A_{230}
RNA	Small molecular weight contamination.	Raised A_{260}/A_{280} ratio
Genomic DNA	High molecular weight contamination, which upon restriction enzyme digestion produces a smear.	Unexpectedly high A_{260}
Denatured supercoiled plasmid	Band that runs faster than supercoiled plasmid and will not itself digest.	-
Nicked linear or open circular plasmid	Band that runs slower than supercoiled plasmid.	-
Oligomeric and multimeric plasmid*	Band that runs slower than supercoiled plasmid, which upon restriction enzyme digestion produces a linear monomeric form.	-
Deletion mutants	Smaller faster migrating species, which upon restriction enzyme digestion show an unexpectedly small size.	-

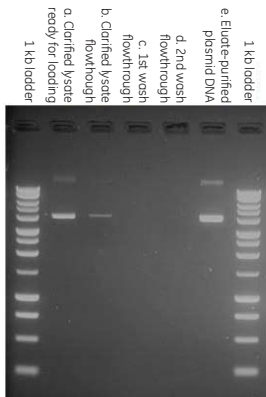
*Multimeric forms do not affect the quality of plasmid DNA and should not be mistaken for genomic DNA.

6.3. Purification analysis

To the samples taken during the Purification step (green arrow image) add an equal volume of Isopropanol. Mix briefly and centrifuge at $16\ 000 \times g$ in a benchtop centrifuge for 10 minutes. Decant or aspirate the supernatant, taking care not to disturb the pellet. Add 1 ml 70% Ethanol and centrifuge again for 5 minutes at $16\ 000 \times g$. Decant or aspirate the supernatant. Air dry the pellet for 3–5 minutes at room temperature, and re-suspend in 20 μ l of TE buffer type 1.

Run 2–5 μ l of each sample on a 1% agarose gel. If possible, assess UV absorbance data with a Nano-Drop spectrophotometer. A typical gel image is shown below.

Figure 2: Typical gel image obtained using samples obtained for Purification analysis.



A 6.3 Kbp high-copy number plasmid was purified from *E. coli* TOP10 using the **illustra plasmidPrep Midi Flow Kit**. Samples are from protocol 5.3 step 4. Purification, at the stages indicated at the top of the gel. RNA may be visible at stages b., c. & d. as a faint low molecular weight band.

6.4. Factors affecting plasmid yield, quality & purity

Certain steps in the isolation and purification of plasmid DNA using the illustra plasmidPrep Midi Flow Kit have been optimized. They are essential for the production of good quality, highly pure plasmid DNA. These steps may be influenced by a number of factors that are detailed below:

Host strains

Many bacterial strains have been developed for the propagation of plasmids and can be used in conjunction with the illustra plasmidPrep Midi Flow Kit. Bacterial strains from the DH series, C600, XL1-blue TOP series, and JM series generally provide good yield and purity of plasmid. However, the following points should be considered:

Oligomeric forms

The replication machinery in certain strains can lead to the production of oligomeric and multimeric forms of plasmid. Oligomeric forms are visible on agarose gels, but are not thought to affect performance in downstream applications.

Endogenous nuclease activity

Certain strains, including early JM and HB series, can have high levels of endogenous nuclease activity, resulting in nicked or re-arranged plasmid.

Carbohydrate levels

Some strains, including TG1 and the JM series, when cultured in rich media can produce high levels of carbohydrate. It is advisable to use simple media such as LB, reduce culture volumes and harvest during late-logarithmic or early stationary phase. Follow protocol in section 5.3 when purifying high or low copy number plasmids.

When purifying very low copy number plasmids, excessive carbohydrates may be produced as a consequence of the larger

culture volumes required. Follow protocol in section 5.4, noting the increase in the volume of Lysis buffers type 11, 12 & 13 to 10 ml for the Lysis step and 3×10 ml washes during the Purification step (for buffer recipes see sections 6.6).

If problems persist, it is advisable to switch bacterial strains.

Inoculation techniques

Always prepare plasmid from a culture that has been cultured from a single colony grown on a fresh selective agar plate. Avoid inoculating straight from liquid cultures, frozen stocks or old plates as this can lead to plasmid re-arrangement, contamination, mutation or total loss of the plasmid.

Always use a starter culture to inoculate the larger cultures as detailed in sections 5.3 & 5.4. This adapts the bacteria for rapid liquid culture, and ensures inoculation with a mid-logarithmic growth phase culture.

Culture medium, volume and growth

The illustra plasmidPrep Midi Flow Kit has been optimized to isolate 100–250 μ g plasmid DNA from cultures containing $3\text{--}9 \times 10^9$ cells/ml. Increasing the cell density or culture volume will not necessarily increase yield or purity and may have a detrimental effect on plasmid quality. The use of too many cells can result in an inefficient Lysis step, leading to reduced yields, ineffective RNA digestion and genomic DNA contamination. Either dilute cultures with cell densities exceeding 9×10^9 cells/ml or use lower culture volumes.

For best results, use a basic culture medium, such as LB broth. The use of rich media such as Terrific Broth (TB) and $2 \times$ YT broth (16 g Tryptone, 10 g Yeast Extract, 5 g Sodium Chloride per liter medium) is not recommended. Cultures should be grown to late logarithmic or early stationary phase, which for most cultures of *E. coli* is 16–20 hours incubation at 37°C, given adequate aeration. Prolonged culture times may result in bacteria entering decline phase, resulting in cell

rupture during incubation, and reduced plasmid yield and purity.

Plasmid characteristics

Copy number

Plasmid copy number depends upon a number of factors, including plasmid size, characteristics of the insert and level of expression, the origin of replication and the host strain. Protocols are provided for working with high, low and very low copy number plasmids; please ensure you follow the correct protocol.

Chloramphenicol (170 µg/ml) may be added to mid-logarithmic phase cultures containing low copy number relaxed plasmids to inhibit protein synthesis and replication of the host genome. The culture is then incubated for a further 4–8 hours during which plasmid replication proceeds unhindered, resulting in an accumulation of plasmid and an increase in copy number. When purifying relaxed plasmids from cultures that have been amplified using chloramphenicol, follow the protocol for high copy number plasmids.

Size


Most large plasmids are low or very low copy number due to the physiological stress that replicating them places upon the cell. Larger plasmids are prone to re-arrangements and mutations, and therefore extra care should be taken to ensure cultures are fresh and harvested in early stationary phase. Larger plasmids are also more susceptible to shear forces, and so vigorous mixing and repeat pipetting should be avoided throughout the protocol (and when using the plasmid in downstream applications).

6.5. Troubleshooting guide

This guide may be helpful in the first instance, however if problems persist or for further information please contact GE Healthcare technical services. Telephone numbers are on the back page. Alternatively log onto <http://www.gelifesciences.com/illustra>

Problem: Low yield of plasmid

Possible cause	Suggestions
<i>Bacterial growth conditions inappropriate</i>	<ul style="list-style-type: none">• To obtain good aeration, use a sterile flask with a volume of at least 4 times the volume of the culture.• Vigorous shaking of the culture is required (225 rpm).• Check media contains appropriate antibiotic selection.
<i>Cell density too high, resulting in poor cell lysis, or column overloading</i>	<ul style="list-style-type: none">• Harvest culture at late-logarithmic or early stationary phase. Expected A_{600} is 1.3–2.6 with $3\text{--}9 \times 10^9$ cells/ml.• Once culture reaches required density, process cells rapidly. Alternatively, store cell pellet at -20°C.
<i>Inefficient lysis with Lysis buffer type 11</i>	<ul style="list-style-type: none">• Ensure cells thoroughly re-suspended in Lysis buffer type 11. The presence of a brown pellet at the end of the Clarification step may indicate inefficient lysis. Expected pellet color after Clarification is predominantly white.

Possible cause	Suggestions
<i>Inadequate mixing of sample with Lysis buffer type 13</i>	<ul style="list-style-type: none"> Once Lysis buffer type 13 has been added to the sample, mix by inversion until a flocculent precipitate appears. Continue inverting until the precipitate is evenly dispersed, the brown color disappears and the solution is less viscous (approximately 6 inversions).
<i>Clarified lysate, Wash buffer type 2 or Elution buffer type 3 not allowed to drain from illustra Fast-Flow plasmid 250 column completely</i>	<ul style="list-style-type: none"> Ensure column is allowed to drain completely before proceeding to the next step.
<i>Pellet loss during Concentration or De-salination steps</i>	<ul style="list-style-type: none"> After the Concentration step, the pellet may be difficult to see. Mark the outside of the high speed centrifuge tube before centrifugation to allow the pellet to be located.  Re-orientate the tube in the centrifuge using this mark for each subsequent spin to avoid disturbing the pellet. Ensure that the supernatant is removed immediately. When decanting supernatant, orient the tube such that the pellet is on the upper-side and slowly decant the supernatant into a sterile container so that any lost pellet can be recovered. Alternatively, use a sterile pipet.

Possible cause	Suggestions
<i>DNA pellet incompletely re-suspended in TE buffer type 1 after De-salination step</i>	<ul style="list-style-type: none"> Wash walls of the centrifuge tube with TE buffer type 1 to ensure maximum yield.
<i>Pellet over dried and failed to dissolve in TE buffer type 1</i>	<ul style="list-style-type: none"> If pellet was over dried, heat the TE buffer type 1 to 60°C prior to addition, or gently roller mix buffer on the pellet overnight using a horizontal roller mixer.

Problem: Genomic DNA contamination (see Table 6)

Possible cause	Suggestions
<i>Cell density too high, resulting in poor cell lysis, or column overloading</i>	<ul style="list-style-type: none"> Check bacterial growth conditions and cell density are correct (For high and low copy number plasmids see Table 4 page 19. For very low copy number plasmids final cell density should be $3-9 \times 10^9$ cells/ml).
<i>Too vigorous mixing of sample after addition of Lysis buffer type 12 and/or 13</i>	<ul style="list-style-type: none"> Mix by inversion only. Do not vortex.
<i>Perceived contamination is multimeric supercoiled plasmid</i>	<ul style="list-style-type: none"> Genomic DNA contamination will appear as large molecular weight contamination on agarose gels that digests to form a smear. A_{260} yield may also be unexpectedly high.

Problem: RNA contamination (see Table 6)

Possible cause	Suggestions
<i>Cell density too high, resulting in poor cell lysis, or column overloading</i>	<ul style="list-style-type: none">• Check bacterial growth conditions and cell density are correct (For high and low copy number plasmids see Table 4 page 19. For very low copy number plasmids final cell density should be $3-9 \times 10^9$ cells/ml).
<i>Lysis buffer type 11 not pre-chilled before use</i>	<ul style="list-style-type: none">• For convenience, store Lysis buffer type 11 at 4°C.• If RNA is evident as small molecular weight contamination on an agarose gel and a raised A_{260}/A_{280} ratio, the plasmid may be treated with fresh RNase I or Lysis buffer type 11, loaded onto an illustra Fast-Flow plasmid 250 column and purified following the protocol from the Purification step f. Wash - Apply 10 ml Wash buffer type 2 onwards.

Problem: Supercoiled plasmid is denatured (see Table 6)

Possible cause	Suggestions
<i>Incubation of sample in Lysis buffer type 12 was longer than 5 minutes</i>	<ul style="list-style-type: none">• Supercoiled plasmid DNA has been irreversibly damaged. It is normal to see levels of up to 5% of plasmid exhibiting this damage. Do not incubate sample in Lysis buffer type 12 for longer than 5 minutes.

Problem: Nicked, linear or open circular plasmid in sample (see Table 6, page 34)

Possible cause	Suggestions
<i>Use of endonuclease containing bacterial strain</i>	<ul style="list-style-type: none">• See section 6.4 for selection of host strain.
<i>Nuclease contamination of buffers</i>	<ul style="list-style-type: none">• Wear gloves and use sterile plastic wear for all steps. Make fresh buffers according to section 6.6.
<i>Use of a different buffer for TE buffer type 1</i>	<ul style="list-style-type: none">• Use the buffer provided in the kit.
<i>Vigorous mixing, vortexing or excessive pipetting</i>	<ul style="list-style-type: none">• Follow advice for mixing given at each step.

Problem: Multimeric plasmid present in sample (see Table 6, page 34)

Possible cause	Suggestions
<i>Selected bacterial strain</i>	<ul style="list-style-type: none">• See section 6.4 for selection of host strain.

**Problem: Deletion mutant plasmid present in sample
(see Table 6)**

Possible cause	Suggestions
<i>Bacterial culture allowed to grow for too long. Agar plate not fresh.</i>	<ul style="list-style-type: none">• Check bacterial growth conditions and cell density are correct (For high and low copy number plasmids see Table 4 p19. For very low copy number plasmids final cell density should be $3-9 \times 10^9$ cells/ml).
<i>Plasmid insert inherently unstable or toxic</i>	<ul style="list-style-type: none">• Prepare plasmid from 4 or more different colonies.
<i>Plasmid re-arrangement (especially in larger constructs)</i>	<ul style="list-style-type: none">• Prepare plasmid from 4 or more different colonies.

Problem: Salt contamination (see Table 6, page 34)

Possible cause	Suggestions
<i>Isopropanol not at room temperature for Concentration step</i>	<ul style="list-style-type: none">• Ensure Isopropanol at room temperature before addition to sample.
<i>Sides of tube not washed with 70% Ethanol for De-salination step</i>	<ul style="list-style-type: none">• Wash sides of tube carefully.• To remove salt, re-precipitate DNA-add 1/10th volume 3 M Sodium Acetate pH 5.4 and 1.1 volumes of Isopropanol. Pellet by centrifugation and wash twice with 70% Ethanol.

Problem: Protein contamination (see Table 6)







Possible cause	Suggestions
<i>Flocculent present in sample after Clarification step. May result in blocked or slow flowing illustra Fast-Flow plasmid 250 column</i>	<ul style="list-style-type: none">• Transfer the supernatant to a fresh tube prior to column loading to avoid transfer of flocculent to column.

Problem: Endotoxin contamination (as measured with Cambrex-1000 kit)

Possible cause	Suggestions
<i>illustra Fast-Flow plasmid 250 column overloaded</i>	<ul style="list-style-type: none">• Cell density greater than $3-9 \times 10^9$ cells/ml can overload the columns and result in endotoxin contamination.
<i>User-introduced endotoxin contamination</i>	<ul style="list-style-type: none">• Use sterile, clean tubes and plasticware throughout.

6.6. Buffer composition

Use nuclease free water and sterile plasticware for buffer preparation. Filter sterilize before use.

Identification	Buffer	Composition
	Resuspension Buffer 1 (Red colored cap)	100 mM Tris/HCl pH 7.5 10 mM EDTA 0.4 mg/ml RNase I
	Lysis buffer type 12 (Clear colored cap)	200 mM NaOH 1% SDS
	Lysis buffer type 13 (Blue colored cap)	3 M KOAC pH 5.5
	Wash buffer type 2 (Yellow colored cap)	50 mM Tris/HCl pH 8 1 mM EDTA 650 mM NaCl
	Elution buffer type 3 (Silver colored cap)	50 mM Tris/HCl pH 8 1 mM EDTA 1.2 M NaCl
	TE buffer type 1 (Green colored cap)	10 mM Tris/HCl pH 8 1 mM EDTA

6.7. Related products

A full range of Molecular Biology reagents can be found in the GE Healthcare catalog or on the web site

<http://www.gelifesciences.com/illustra>

If you need further information, GE technical services are happy to assist (world-wide phone numbers can be found on the back cover).

Application	Product	Product code	Pack sizes
Convenient buffer addition to Fast-Flow 250 plasmid column	LabMate PD10 Buffer Reservoir	18-3216-03	10
Preparation of additional buffers	Water, nuclease-free	US70783	500 ml
Rapid extraction and purification of plasmid DNA from small scale cultures of <i>E.coli</i>	illustra plasmidPrep Mini Spin Kit	28-9042-69 28-9042-70	50 purifications 250 purifications
Purification of DNA from PCR and enzymes; multiple samples	illustra GFX™ PCR DNA and Gel Band Purification Kit	28-9034-70 28-9034-71	100 purifications 250 purifications
DNA ligation	DNA ligation system	RPN1507	50 reactions
Blunt-Ended PCR Cloning	Blunt-ended PCR Cloning Kit	RPN5110	40 reactions
Sequencing reaction kit optimized for MegaBACE™ DNA analysis system	DYEnamic™ ET terminator cycle sequencing kit	US781050	100 templates

Application	Product	Product code	Pack sizes
Purification of oligonucleotides and 10–50 bp DNA fragments	illustra MicroSpin™ G-25 Columns (100-150 ml sample volume)	27-5325-01	50 purifications
Purification of oligonucleotides following synthesis	illustra NAP™-5 Columns	17-0853-01	50 purifications
Preparation of PCR products for automated sequencing	ExoSAP-IT™	US78200	100 reactions
Dye terminator removal from automated sequencing reactions	illustra AutoSeq™ G-50 Dye Terminator Removal Kit	27-5340-01	50 purifications

7. References

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3. Sambrook, J., Fritsch, E.F. & Maniatis, T., *Molecular cloning: A laboratory Manual*, Cold Spring Harbor laboratory, 2nd eds., (1989).
4. Vogelstein, B. & Gillespie, D., *Proc. Natl. Acad. Sci. USA* 76, 615 (1979).
5. Marko, M.A., Chipperfield, R. & Birnboim, H.C., *Anal. Biochem.* 121, 382 (1982).
6. Ausubel, F.M. et al., eds., *Current Protocols in Molecular Biology* 1, 1.68 (1991).
7. Voo, K.S. & Jacobsen B.M., (1998), *Biotechniques*, 24, 240-243 (1998).

GE Healthcare offices:

GE Healthcare Bio-Sciences AB
Björkgatan 30 751 84, Uppsala
Sweden

GE Healthcare Europe GmbH
Munzinger Strasse 5 D-79111, Freiburg,
Germany

GE Healthcare Bio-Sciences Corp
800 Centennial Avenue, P.O. Box 1327,
Piscataway, NJ 08855-1327,
USA

GE Healthcare Bio-Sciences KK
Sanken Bldg. 3-25-1, Hyakunincho,
Shinjuku-ku, Tokyo 169-0073,
Japan

For contact information for your local office,
please visit: www.gelifesciences.com/contact

GE Healthcare UK Limited
Amersham Place, Little Chalfont, Buckinghamshire,
HP7 9NA, UK

<http://www.gehealthcare.com/lifesciences>

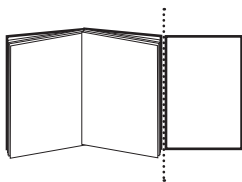


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28-9042-66PL AE 05/2009

The next two pages are a
protocol card.
Please add to the back page as a
tear off addition.



Quick Reference Protocol Card

28-9042-67 (25 purifications)
28-9042-68 (100 purifications)






illustra™ plasmidPrep Midi Flow Kit

Protocol for purification of high copy number and low copy number plasmid DNA







- Ensure **Lysis buffer type 12** does not contain precipitate-warm to dissolve.
- Pre-chill **Lysis buffer type 13** to 4°C. • Ensure suitable high speed centrifuge tubes are available.

 :Add  :Spin  :Incubate

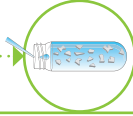
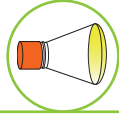
1. Bacterial culture preparation

-  Inoculate 5 ml starter culture
-  6–8 hours 37°C, 225 rpm
-  Inoculate 50 ml (high copy number plasmids) or 150 ml (low copy number plasmids) culture
-  12–18 hours 37°C, 225 rpm
-  15 minutes 5 000 x g 4°C; discard supernatant

2. Lysis

-  6 ml Lysis buffer type 11; re-suspend pellet thoroughly
-  Transfer sample to high speed polycarbonate or polypropylene centrifuge tube
-  6 ml Lysis buffer type 12; invert to mix
-  5 minutes room temperature
-  6 ml Lysis buffer type 13; invert to mix
-  Incubate 15 minutes on ice

3. Clarification





© 30 minutes 20 000 x g 4°C; retain supernatant

Preparation of illustra Fast-Flow plasmid 250 Column

- Remove top cap and bottom tip
- Allow storage buffer to elute completely



4. Purification

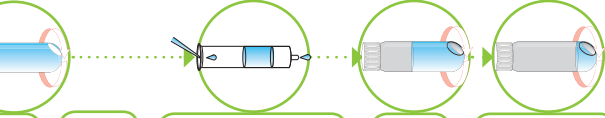
- Load clarified lysate onto column; empty column by gravity flow
-  10 ml Wash buffer type 2; empty column by gravity flow
- Repeat for a total of 2 washes
- Place suitable polyallomer or polypropylene centrifuge tube under column
-  4 ml Elution buffer type 3
- Collect eluate by gravity flow

5. Concentration

-  4 ml Isopropanol to eluate; mix by inversion
-  30 minutes 15 000 x g 4°C; carefully remove supernatant

6. De-salination

- Wash plasmid pellet 5 ml 70% Ethanol
-  10 minutes 15 000 x g 4°C; carefully remove supernatant
- Air dry pellet 5–10 minutes
-  1 ml TE buffer type 1 to dissolve plasmid DNA



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GE Healthcare UK Limited,
Amersham Place, Little Chalfont,
Buckinghamshire, HP7 9NA UK

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