USER GUIDE

invitrogen

PureLink™ HiPure Expi Megaprep and Gigaprep Kit

For purifying large quantities of high-quality plasmid DNA

Catalog Numbers K210008XP, K210009XP

Revision A.0

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TRADEMARKS

Contents

Product Information

Kit contents and storage

This manual is supplied with the following products:

Product	Quantity	Catalog no.
PureLink™ HiPure Expi Mega Kit	4 preps	K210008XP
PureLink™ HiPure Expi Giga Kit	2 preps	K210009XP

Shipping and storage

All components of the PureLink™ HiPure Expi Megaprep and Gigaprep Kits are shipped at room temperature.

Upon receipt, store all components at room temperature.

Contents

The components included in the PureLink™ HiPure Expi Megaprep and Gigaprep Kits are listed below.

Component	Megaprep	Gigaprep
Cat. No.	K210008XP	K210009XP
Resuspension Buffer (R3)	250 mL	250 mL
RNase A	1.5 mL	1.5 mL
Lysis Buffer (L7)	250 mL	250 mL
Precipitation Buffer (N3)	200 mL	250 mL
Equilibration Buffer (EQ1)	400 mL	400 mL
Wash Buffer (W8)	4 × 400 mL	3 × 400 mL
Elution Buffer (E4)	400 mL	400 mL
TE Buffer (TE)	30 mL	30 mL
Lysate Filtration Columns	4 each	2 each
DNA-Binding Cartridge	4 each	2 each

Buffer composition

The composition of buffers included in the PureLink™ HiPure Expi Megaprep and Gigaprep Kits is listed below.

Buffer	Composition
Resuspension Buffer (R3)	50 mM Tris-HCl, pH 8.0 10 mM EDTA
RNase A	20 mg/mL in 10 mM Sodium acetate, pH 5.2
Lysis Buffer (L7)	0.2 M NaOH 1% (w/v) SDS
Precipitation Buffer (N3)	3.1 M Potassium acetate, pH 5.5
Equilibration Buffer (EQ1)	0.1 M Sodium acetate, pH 5.0 0.6 M NaCl 0.15% (v/v) Triton® X-100
Wash Buffer (W8)	0.1 M Sodium acetate, pH 5.0 825 mM NaCl
Elution Buffer (E4)	100 mM Tris-HCl, pH 8.5 1.25 M NaCl
TE Buffer (TE)	10 mM Tris-HCl, pH 8.0 0.1 mM EDTA

Description of the System

About the kit

Use the PureLink™ HiPure Expi Megaprep and Gigaprep Kits to isolate large (milligram) quantities of high-purity transfection-grade plasmid DNA using an enhanced anion exchange resin. The kits include vacuum filtration columns that provide bacterial filtration without centrifugation, enabling extremely fast process times.

The PureLink™ HiPure Expi Megaprep Kit typically isolates 4 mg of high quality, ultrapure plasmid DNA with inherently low endotoxin levels from 500 mL of bacterial culture.

The PureLink™ HiPure Expi Gigaprep Kit typically isolates 12 mg of high quality, ultrapure plasmid DNA with inherently low endotoxin levels from 2.5 L of bacterial culture.

Advantages of the PureLink™ HiPure Expi Megaprep and Gigaprep Kits include:

- **High Yield**

  Isolate over 5 mg of high quality plasmid DNA from a single megaprep purification using 1 L of bacterial culture volume.

  Isolate up to 14 mg of high quality plasmid DNA from a single gigaprep purification using 5L of bacterial culture volume.

- **Purity**

  Low endotoxin levels (typically 0.1–1.0 EU/µg), and A_{260}/A_{280} >1.8, making it ideal for mammalian cell transfection.

- **Simple and super-fast protocol**

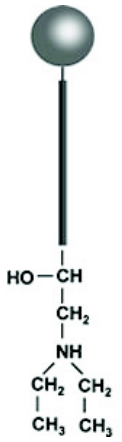
  With vacuum assisted protocol, typical plasmid isolation takes 90 minutes.

System overview

To isolate plasmid DNA using the kit, grow transformed *E. coli* cells overnight and harvest them with a centrifuge. You resuspend the cells in Resuspension Buffer (R3) with RNase, and then lyse the cells with Lysis Buffer (L7). You add Precipitation Buffer (N3) to the lysate and clarify the lysate by passing it over a pre-packed Lysate Binding Cartridge. Then, you pass the cleared lysate over a pre-packed DNA-Binding Cartridge.

The negatively charged phosphates on the DNA backbone interact with the positive charges on the resin surface of the DNA-Binding Cartridge. DNA binding is influenced by temperature, salt concentration, and the pH of the solution. Under moderate salt conditions, the plasmid DNA binds to the resin of the DNA-Binding Cartridge. RNA, proteins, carbohydrates and other impurities are washed away using the Wash Buffer (W8). The plasmid DNA is then eluted under high salt conditions with Elution Buffer (E4). The eluted DNA is desalted and concentrated with an alcohol precipitation step.

HiPure technology



The HiPure technology is based on next generation anion-exchange chromatography. The technology uses a patented resin composed of small particles with a uniform pore size, to provide high yields and reproducible performance.

The spacer arm with increased length provides improved DNA binding efficiency. The unique patented ion-exchange moiety provides high efficiency for separation of DNA from cellular contaminants including RNA.

System specifications

Specifications*	Megaprep	Gigaprep
Starting *E. coli* Culture Volume at OD 2.0	500 mL	2.5 L
Binding Capacity**	5 mg	14 mg
Lysate Filtration Cartridge Reservoir Capacity	230 mL	450 mL
DNA-Binding Cartridge Reservoir Capacity	200 mL	400 mL
Elution Volume	50 mL	100 mL
DNA Yield***	4 mg	12 mg

* Specifications and results are based on high copy number plasmids.

** Binding capacity varies with plasmid copy number, type and size, and volume of bacterial culture used.

***DNA yield depends on plasmid copy number, type and size; bacterial strain; and growth conditions.

Downstream applications

Plasmid DNA isolated using the PureLink™ HiPure Expi Megaprep and Gigaprep Kits is suitable for use in a variety of downstream applications including:

- Mammalian transfection
- Automated fluorescent or manual DNA sequencing
- PCR cloning
- *in vitro* transcription
- Restriction digestion

Methods

Before Starting

Introduction

Guidelines are included for growing the overnight bacterial cell culture and for determining the appropriate amounts of starting material based on the plasmid copy number used.

Note

Some of the buffers in the PureLink™ HiPure Expi Megaprep and Gigaprep Kits contain hazardous chemicals. For your protection, always wear a laboratory coat, disposable gloves, and eye protection when handling buffers.

IMPORTANT! To avoid the possibility of implosion, do not use vessels that are not designed for use with vacuum. Do not use vessels that are cracked or scratched. Always wear safety glasses when working near a bottle or flask under vacuum.

Grow bacterial cultures

Grow transformed *E. coli* cells overnight in Luria-Bertani (LB) medium with the appropriate antibiotic. The bacterial culture should have a cell density of approximately 10^9 cells/mL or an absorbance of 1–1.5 at 600 nm (A_{600}). Use a bacterial culture in stationary phase.

Plasmid type and copy number

Use a high copy-number plasmid to obtain a good yield of plasmid DNA. High copy number plasmids typically yield 2–6 µg DNA/mL LB culture with an A_{600} of 2.0. Typical yields from low copy number plasmids are highly dependent upon culture conditions and vector/host strain combinations.

If you are using a low copy-number plasmid, use a higher volume of LB culture, as directed in the protocol.

The table below lists the volumes of LB culture required for the Megaprep and Gigaprep procedures depending on the plasmid copy number used:

Plasmid Copy Number	Megaprep	Gigaprep
High copy number plasmid	500 mL	2.5 L
Low copy number plasmid	2.5 L	5 L

General guidelines

Follow the recommendations below to obtain the best results:

- Maintain a sterile environment when handling DNA to avoid any contamination from DNases.
- Ensure that no DNase is introduced into the sterile solutions supplied with the kits.
- Make sure all equipment that comes in contact with DNA is sterile, including pipette tips and tubes.
- Save aliquots as recommended during the procedure. The aliquots allow you to troubleshoot (see page 20).
- Use TE Buffer provided in the kit or 10 mM Tris-HCl, pH 8.0 to resuspend the DNA pellet.

Prepare Buffers

Resuspension Buffer (R3)

Add RNase A to the Resuspension Buffer (R3) according to instructions on the bottle label. Mix well. Mark the bottle label to indicate that RNase A is added. Store Resuspension Buffer with RNase at 4°C.

Lysis Buffer (L7)

Check the Lysis Buffer (L7) for precipitates. If present, warm the solution in a 37°C water bath for 5 minutes to dissolve the precipitate.

Set up the Lysate Filtration Cartridge

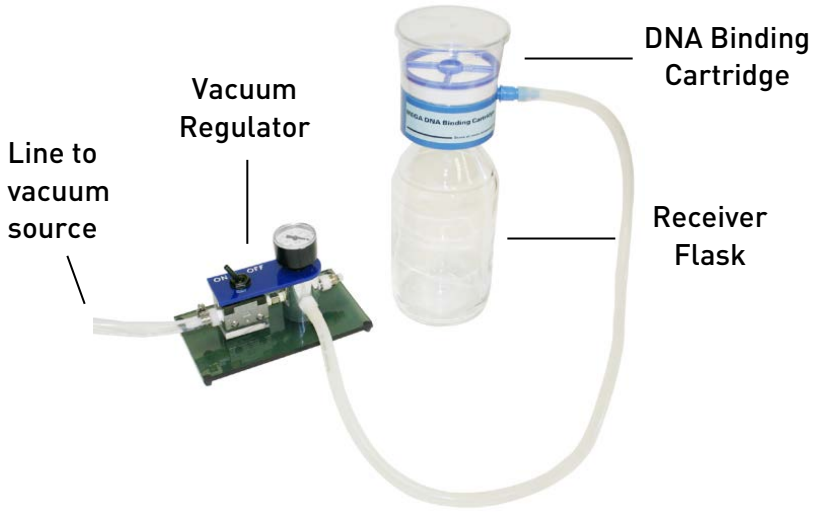
Attach the Megaprep or Gigaprep Lysate Filtration Cartridge onto a clean 1000-mL Stericup® Receiver flask or equivalent.

Connect the vacuum source to the tubing connector of the filtration cartridge. Examples of the setups are shown in the figures below.

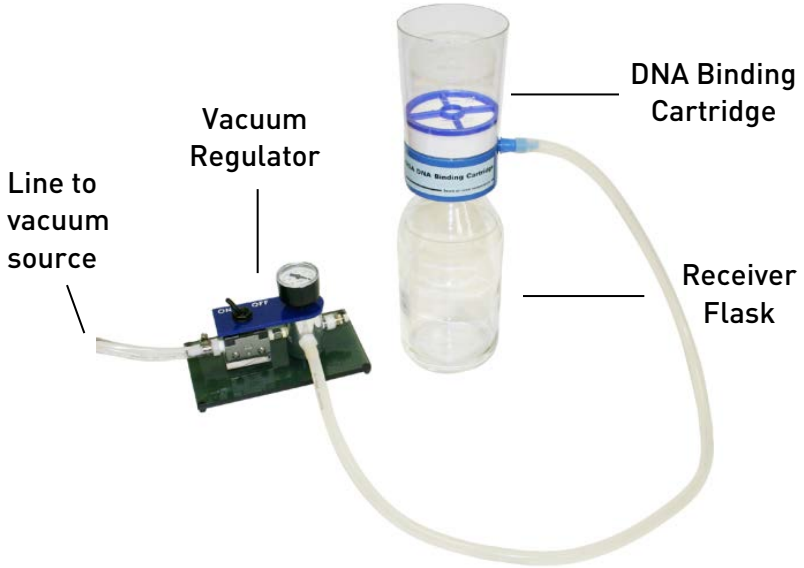
Make sure that the vacuum source has a vacuum regulator to adjust the vacuum pressure during the procedure.

Note: Do not over-tighten the filtration cartridge on the bottleneck or the filtration cartridge plastic may crack.

Megaprep DNA-Binding Cartridge setup



Gigaprep DNA-Binding Cartridge setup



Megaprep Procedure

Introduction

The PureLink™ HiPure Expi Megaprep procedure can be completed in 90 minutes using 500 mL overnight cultures containing high copy number plasmids, or 2.5 L of overnight cultures containing low copy number plasmids.

For instructions on performing the PureLink™ HiPure Expi Gigaprep procedure, see page 14.

Before starting

Verify that RNase A is added to the Resuspension Buffer (R3). Warm Lysis Buffer (L7) briefly at 37°C to redissolve any particulate matter. See page 9 for details.

Materials needed

- Overnight culture of transformed *E. coli* cells (0.5–2.5 L, see page 8 for details on volumes for low and high copy number plasmids)
- Isopropanol
- 70% ethanol
- Buckets, rotor, and centrifuge for harvesting cells
- Sterile 30-mL centrifuge tubes
- Sterile spatula
- Sterile container for transferring the lysate
- Centrifuge and rotor capable of reaching >12,000 × *g* at room temperature and 4°C
- Vacuum source capable of generating a negative pressure of –600 to –800 mbar and equipped with a vacuum regulator.
- 1000-mL Stericup® Receiver flask with 45-mm thread (Millipore Cat. no. SC00B10RE) or a sterile 1-L glass bottle
- 250-mL Stericup® Receiver flask with 45 mm thread (Millipore cat. no. SC00B02RE) or equivalent

Components supplied with the kit

- Resuspension Buffer (R3) with RNase A (page 9)
- Lysis Buffer (L7)
- Precipitation Buffer (N3)
- Equilibration Buffer (EQ1)
- Wash Buffer (W8)
- Elution Buffer (E4)
- TE Buffer (TE)
- Megaprep Lysate Filtration Cartridge
- Megaprep DNA-Binding Cartridge

Prepare cell lysate

1. For **high copy number plasmids**, use up to 500 mL of an overnight LB culture per sample.

   For **low copy number plasmids**, use up to 2.5 L of an overnight LB culture per sample.

2. Harvest the cells by centrifuging the overnight LB culture at 4000 × *g* for 15 minutes. Remove all medium.

3. Add 50 mL Resuspension Buffer (R3) with RNase A to the pellet and resuspend the cells until homogeneous. No cell clumps should be visible.

4. Add 50 mL Lysis Buffer (L7). Mix by gently shaking until the lysate mixture is thoroughly homogenous. **Do not vortex.** Incubate the tube at room temperature for 5 minutes.

   Note: The presence of genomic DNA causes the mixture to be very viscous at this stage. Do not vortex the cell suspension because vortexing may cause genomic DNA shearing.

5. Add 50 mL Precipitation Buffer (N3) and mix gently shaking until the mixture is thoroughly homogeneous. **Do not vortex**. A white, flocculent precipitate of proteins, cellular debris, genomic DNA, and detergent forms.

   Note: The neutralized lysate must be completely non-viscous, without any remnant of the viscous matter present after cell lysis (step 4).

6. Pour the bacterial lysate from step 5 directly into the prepared Megaprep Lysate Filtration Cartridge (see page 9). Incubate the cartridge at room temperature for **at least 2 minutes** without agitation.

   Note: It is very important to let the lysate stand for at least 2 minutes in the cartridge to allow the precipitate to float and form a layer on top of the lysate, to prevent clogging and ensure efficient filtration.

7. Make sure that the vacuum source is connected to the tubing connector of the filtration cartridge and apply the vacuum (−600 to −800 mbar). Collect the clear flow-through into the flask. Keep the vacuum on until all of the liquid has drained, then turn off the vacuum source.

   Note: An unclogged column filters 125 mL of filtrate in 1–2 minutes when vacuum is applied.

8. Add 50 mL Wash Buffer (W8) to the filtration cartridge and gently stir the precipitate with a sterile spatula.

   Note: Gentle agitation of the precipitate improves the flow of liquid through the filter unit.

9. Apply the vacuum until all of the liquid has drained from the filtration cartridge.

   The receiving flask contains the clarified lysate with the plasmid DNA.

10. Remove and discard the filtration cartridge into biohazardous waste. Proceed to **Bind and wash DNA** (page 12).

Bind and wash DNA

1. Attach the Megaprep DNA-Binding Cartridge onto the 1000-mL Stericup® Receiver flask or equivalent.
2. Add 100 mL Equilibration Buffer (EQ1) to the binding cartridge. Apply the vacuum through tubing connected to the binding cartridge connector and keep the vacuum on until all of the liquid has drained from the binding cartridge. Then, turn off the vacuum and discard the flow-through.
3. Load the filtered lysate from **Prepare cell lysate**, step 9 into the equilibrated Megaprep DNA-Binding Cartridge and apply vacuum until all of the lysate has passed through the binding cartridge.

   Save a 100-µL aliquot of the flow-through for further analysis.
4. Add 175 mL Wash Buffer (W8) to the cartridge and apply the vacuum until all of the liquid has drained from the cartridge. Turn off the vacuum.

   Save a 100 µL aliquot of the flow-through for further analysis.
5. **Repeat** step 4, once. Discard the flow-through.

   The binding cartridge contains the DNA.
6. Proceed to **Elute DNA**.

Elute DNA

1. Remove the Megaprep DNA-Binding Cartridge (containing the DNA) from the 1000-mL Stericup® Receiver flask and attach the binding cartridge onto a clean, sterile 250-mL Stericup® Receiver flask.
2. Add 50 mL Elution Buffer (E4) to the binding cartridge.
3. Apply a *soft* vacuum (−200 to −300 mbar) through the tubing attached to the binding cartridge connector. Apply the vacuum until approximately 10–20 mL of Elution Buffer (E4) has passed through the cartridge. Then, turn off the vacuum.
4. Let the cartridge stand for 1 minute without agitation.
5. Apply the *soft* vacuum again until all of the Elution Buffer (E4) has drained from the binding cartridge into the Stericup® Receiver flask.
6. (*Optional*) Elution: Add an additional 50 mL Elution Buffer (E4) to the binding cartridge. Repeat steps 3–5.

   Note: This second elution step can increase the final DNA yield by approximately 10%.

   The 250-mL Receiver flask now contains the eluate with the purified DNA.

   Save a 100 µL aliquot of the eluate for further analysis.
7. Proceed to **Precipitate DNA** (page 13).

Precipitate DNA

1. Transfer all of the eluate from step 5 or 6 (above) into several 30-mL centrifuge tubes (maximum 17.5 mL per tube).
2. Add 0.7 volume isopropanol per volume of eluate.
3. Centrifuge the tubes >12,000 × *g* for 30 minutes at 4°C.

   Note: Use a centrifuge with a swinging bucket rotor that allows >12,000 × *g* to prevent the plasmid DNA from sticking to the walls of the centrifuge tube. If such a rotor is not available, siliconize the centrifuge tubes with a repellent silane (dimethyldichlorosilane).
4. Discard the supernatant. Resuspend the pellet in 20 mL of 70% ethanol to wash the DNA. Vortex gently.
5. Centrifuge at >12,000 × *g* for 10 minutes at 4°C.
6. Air-dry the DNA pellet for 10 minutes.
7. Resuspend the pellet in 2–3 mL of TE Buffer (TE).

Store DNA

Store the purified DNA at 4°C for immediate use or aliquot the DNA and store at –20°C for long-term storage. Avoid repeated freezing and thawing of DNA.

Note

DNA dissolves best under alkaline conditions; it does not easily dissolve in acidic buffers. Ensure that the pH of the buffer used for redissolving is ≥8.0

Air-dry pellet instead of using a vacuum, especially if the DNA is of high molecular weight.

Redissolve DNA by warming the solution slightly

Allowing more time for redissolving.

Increase volume of buffer used for redissolving if necessary if the solution above the pellet is highly viscous.

Gigaprep Procedure

Introduction

The PureLink™ HiPure Expi Gigaprep procedure can be completed in 90 minutes using 2.5 L overnight cultures containing high copy number plasmids, or 5 L of overnight cultures containing low copy number plasmids.

For instructions on performing the PureLink™ HiPure Expi Megaprep procedure, see page 10.

Before starting

Verify that RNase A is added to the Resuspension Buffer (R3). Warm Lysis Buffer (L7) briefly at 37°C to redissolve any particulate matter. See page 9 for details.

Materials needed

- Overnight culture of transformed *E. coli* cells (2.5–5 L, see page 8 for details on volumes for low and high copy number plasmids)
- Isopropanol
- 70% ethanol
- Buckets, rotor, and centrifuge for harvesting cells
- Sterile 30-mL centrifuge tubes
- Sterile spatula
- Sterile container for transferring the lysate
- Centrifuge and rotor capable of reaching >12,000 × *g* at room temperature and 4°C
- Vacuum source capable of generating a negative pressure of –600 to –800 mbar and equipped with a vacuum regulator.
- 1000-mL Stericup® Receiver flask with 45-mm thread (Millipore Cat. no. SC00B10RE) or a sterile 1-L glass bottle
- 250-mL Stericup® Receiver flask with 45 mm thread (Millipore cat. no. SC00B02RE) or equivalent

Components supplied with the kit

- Resuspension Buffer (R3) with RNase A (page 9)
- Lysis Buffer (L7)
- Precipitation Buffer (N3)
- Equilibration Buffer (EQ1)
- Wash Buffer (W8)
- Elution Buffer (E4)
- TE Buffer (TE)
- Gigaprep Lysate Filtration Cartridge
- Gigaprep DNA-Binding Cartridge

Prepare cell lysate

1. For **high copy number plasmids**, use up to 2.5 L of an overnight LB culture per sample.

   For **low copy number plasmids**, use up to 5 L of an overnight LB culture per sample.

2. Harvest the cells by centrifuging the overnight LB culture at 4000 × *g* for 15 minutes. Remove all medium.

3. Add 125 mL Resuspension Buffer (R3) with RNase A to the pellet and resuspend the cells until homogeneous. No cell clumps should be visible.

4. Add 125 mL Lysis Buffer (L7). Mix by gently shaking until the lysate mixture is thoroughly homogenous. **Do not vortex.** Incubate the tube at room temperature for 5 minutes.

   Note: The presence of genomic DNA causes the mixture to be very viscous at this stage. Do not vortex the cell suspension because vortexing may cause genomic DNA shearing.

5. Add 125 mL Precipitation Buffer (N3) and mix gently shaking until the mixture is thoroughly homogeneous. **Do not vortex**. A white, flocculent precipitate of proteins, cellular debris, genomic DNA, and detergent forms.

   Note: The neutralized lysate must be completely non-viscous, without any remnant of the viscous matter present after cell lysis (step 4).

6. Pour the bacterial lysate from step 5 directly into the prepared Gigaprep Lysate Filtration Cartridge (see page 9). Incubate the cartridge at room temperature for **at least 5 minutes** without agitation.

   Note: It is very important to let the lysate stand for at least 5 minutes in the cartridge to allow the precipitate to float and form a layer on top of the lysate, to prevent clogging and ensure efficient filtration.

7. Make sure that the vacuum source is connected to the tubing connector of the filtration cartridge and apply the vacuum (−600 to −800 mbar). Collect the clear flow-through into the flask. Keep the vacuum on until all of the liquid has drained, then turn off the vacuum source.

   Note: An unclogged column filters 125 mL of filtrate in 1–2 minutes when vacuum is applied.

8. Add 50 mL Wash Buffer (W8) to the filtration cartridge and gently stir the precipitate with a sterile spatula.

   Note: Gentle agitation of the precipitate improves the flow of liquid through the filter unit.

9. Apply the vacuum until all of the liquid has drained from the filtration cartridge.

   The receiving flask contains the clarified lysate with the plasmid DNA.

10. Remove and discard the filtration cartridge into biohazardous waste. Proceed to **Bind and wash DNA** (page 16).

Bind and wash DNA

1. Attach the Gigaprep DNA-Binding Cartridge onto the 1000-mL Stericup® Receiver flask or equivalent.
2. Add 200 mL Equilibration Buffer (EQ1) to the binding cartridge. Apply the vacuum through tubing connected to the binding cartridge connector and keep the vacuum on until all of the liquid has drained from the binding cartridge. Then, turn off the vacuum and discard the flow-through.
3. Load the filtered lysate from **Prepare cell lysate**, step 9 into the equilibrated Gigaprep DNA-Binding Cartridge and apply vacuum until all of the lysate has passed through the binding cartridge.

   Save a 100-µL aliquot of the flow-through for further analysis.
4. Add 275 mL Wash Buffer (W8) to the cartridge and apply the vacuum until all of the liquid has drained from the cartridge. Turn off the vacuum.

   Save a 100 µL aliquot of the flow-through for further analysis.
5. **Repeat** step 4, once. Discard the flow-through.

   The binding cartridge contains the DNA.
6. Proceed to **Elute DNA**.

Elute DNA

1. Remove the Gigaprep DNA-Binding Cartridge (containing the DNA) from the 1000-mL Stericup® Receiver flask and attach the binding cartridge onto a clean, sterile 250-mL Stericup® Receiver flask.
2. Add 100 mL Elution Buffer (E4) to the binding cartridge.
3. Apply a *soft* vacuum (−200 to −300 mbar) through the tubing attached to the binding cartridge connector. Apply the vacuum until approximately 30–40 mL of Elution Buffer (E4) has passed through the cartridge. Then, turn off the vacuum.
4. Let the cartridge stand for 1 minute without agitation.
5. Apply the *soft* vacuum again until all of the Elution Buffer (E4) has drained from the binding cartridge into the Stericup® Receiver flask.
6. (*Optional*) Elution: Add an additional 100 mL Elution Buffer (E4) to the binding cartridge. Repeat steps 3–5.

   Note: This second elution step can increase the final DNA yield by approximately 10%.

   The 250-mL Receiver flask now contains the eluate with the purified DNA.

   Save a 100 µL aliquot of the eluate for further analysis.
7. Proceed to **Precipitate DNA** (page 17).

Precipitate DNA

1. Transfer all of the eluate from step 5 or 6 (above) into several 30-mL centrifuge tubes (maximum 17.5 mL per tube).
2. Add 0.7 volume isopropanol per volume of eluate.
3. Centrifuge the tubes >12,000 × *g* for 30 minutes at 4°C.

   Note: Use a centrifuge with a swinging bucket rotor that allows >12,000 × *g* to prevent the plasmid DNA from sticking to the walls of the centrifuge tube. If such a rotor is not available, siliconize the centrifuge tubes with a repellent silane (dimethyldichlorosilane).
4. Discard the supernatant. Resuspend the pellet in 20 mL of 70% ethanol to wash the DNA. Vortex gently.
5. Centrifuge at >12,000 × *g* for 10 minutes at 4°C.
6. Air-dry the DNA pellet for 10 minutes.
7. Resuspend the pellet in 2–3 mL of TE Buffer (TE).

Store DNA

Store the purified DNA at 4°C for immediate use or aliquot the DNA and store at –20°C for long-term storage. Avoid repeated freezing and thawing of DNA.

Note

DNA dissolves best under alkaline conditions; it does not easily dissolve in acidic buffers. Ensure that the pH of the buffer used for redissolving is ≥8.0

Air-dry pellet instead of using a vacuum, especially if the DNA is of high molecular weight.

Redissolve DNA by warming the solution slightly

Allowing more time for redissolving.

Increase volume of buffer used for redissolving if necessary if the solution above the pellet is highly viscous.

Estimating DNA Yield and Quality

Introduction

After isolating DNA, determine the quantity and quality of the purified DNA.

DNA yield

Measure the DNA concentration using UV absorbance at 260 nm or Qubit™ DNA Assay Kits.

UV Absorbance

1. Prepare a dilution of the DNA solution in 10 mM Tris-HCl, pH 7.5. Mix well. Measure the absorbance at 260 nm (A_{260}) of the dilution (using a cuvette with an optical path length of 1 cm) in a spectrophotometer blanked against 10 mM Tris-HCl, pH 7.5.
2. Calculate the concentration of DNA using the formula:

$$\text{DNA } (\mu g/\text{mL}) = A_{260} \times 50 \text{ x dilution factor}$$

For DNA, A_{260} = 1 for a 50 µ*g*/mL solution measured in a cuvette with an optical path length of 1 cm.

Note: Contaminating RNA inflates the DNA content measured at 260 nm. To avoid any interference from RNA, use the Qubit™ DNA Assay Kits for measuring DNA concentration.

Qubit™ DNA Assay Kits

The Qubit™ DNA Assay Kits (see page 22 for ordering information) provide a rapid, sensitive, and specific method for measuring dsDNA concentration with minimal interference from RNA, protein, ssDNA (primers), or other common contaminants that affect UV absorbance.

The kit contains a state-of-the-art quantitation reagent, pre-diluted standards for standard curve, and a pre-made buffer. The assay is designed for reading in standard fluorescent readers/fluorometer or Qubit™ 2.0 Fluorometer.

DNA quality

Typically, DNA isolated using PureLink™ HiPure Expi Megaprep and Gigaprep Kits have an A_{260}/A_{280} ratio >1.8 when samples are diluted in Tris-HCl pH 7.5, indicating that the DNA is substantially clean of proteins that could interfere with downstream applications.

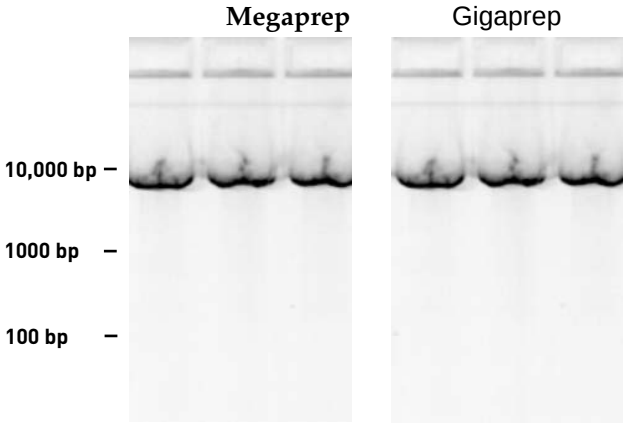
Absence of contaminating RNA may be confirmed by agarose gel electrophoresis or by performing the Qubit™ RNA assay.

Plasmid DNA isolated with the PureLink™ HiPure Expi Megaprep and Gigaprep Kits are mainly in supercoiled form and appears as one major band on agarose gel.

Expected Results

Results

Plasmid DNA (a 7.6 kbp derivative of pcDNA3.3-TOPO, harboring a gene coding for a human heavy human IgG chain) was isolated in triplicate from cultures of *E. coli* (TOP10) using the PureLink™ HiPure Expi Megaprep and Gigaprep Kits as described in this manual. The purified plasmid DNA was analyzed for yield, endotoxin levels, A_{260}/A_{280} ratio, sequencing, restriction enzyme digestion and gel electrophoresis (5 µg) on a 0.8% E-Gel™ EX agarose gel (see below).



5 µg plasmid DNA from a 1 L culture Megaprep procedure or 5 L Gigaprep procedure. The gel shows only supercoiled DNA, with no evidence of RNA contamination.

Summary of expected results

The summary of results using the PureLink™ HiPure Plasmid DNA Megaprep and Gigaprep Purification Kits is listed in the table below.

Note: DNA yield depends on plasmid copy number and type, bacterial strain, and growth conditions.

Results For:	Megaprep	Gigaprep
Processing Time	90 minutes	90 minutes
Plasmid DNA Yield	4 mg	12 mg
Endotoxin Levels*	0.1–1.0 EU/µg	0.1–1.0 EU/µg
Sequencing	Successful	Successful
Restriction Enzyme Digestion	Successful	Successful

* These are typical endotoxin values. Endotoxin levels can vary depending on the bacterial strain, mass of the bacterial pellet, and plasmid.

Troubleshooting

Collected aliquots

If problems arise during the procedure, analyze the aliquots harvested during the procedure on a 1% agarose gel to determine the presence of DNA in the aliquots. The table below lists the normal and abnormal results and probable cause.

Aliquot	Normal Results	Abnormal Results	Cause of Abnormal Results
DNA-Binding Cartridge flow-through	No DNA	DNA	Lysate at improper pH or salt concentration for plasmid DNA to bind column. Cartridge overloaded. DNA-Binding Cartridge damaged.
DNA-Binding Cartridge Wash	No DNA	DNA	DNA-Binding Cartridge damaged.
DNA-Binding Cartridge Eluate	DNA	No DNA	Incorrect pH or salt concentration to release plasmid DNA from column. Use correct Elution Buffer (E4) and perform optional elution step.

Observation	Cause	Solution
Low plasmid DNA yield	Temperature of Lysis Buffer (L7) or Elution Buffer (E4) is too low	Store Lysis Buffer (L7) or Elution Buffer (E4) at room temperature.
	Low copy-number plasmid	Increase the starting culture volume (see page 8).
	Lysate is at improper pH or salt oncentration to bind column	• Carefully remove all culture medium before resuspending cells. • Make sure that the correct volume of Precipitation Buffer (N3) is added.
	DNA-Binding Cartridge is damaged	Do not damage the cartridge during the procedure by overtightening the cartridge or dropping it.
	No DNA precipitated (DNA is present in eluate aliquot but little or no DNA is recovered after precipitation)	• Measure correctly the volume of eluate in each centrifugation tube and add exactly 0.7 volume of isopropanol. • Pellet plasmid DNA at the appropriate speed and temperature in a swinging rotor.
	Plasmid DNA pellet is over-dried	Air-dry the DNA pellet. Do not dry the DNA pellet with a vacuum system.
Cartridge clogged	Cartridge is clogged by lysate	Let the lysate stand in the cartridge to allow the precipitate to float and form a layer on top of the lysate.

Observation	Cause	Solution
Genomic DNA contamination	Genomic DNA sheared during handling	Gently invert tubes to mix after adding buffers L7 and N3. **Do not vortex** as it can shear genomic DNA.
Additional plasmid forms present	Plasmid DNA permanently denatured (band migrating faster than supercoiled DNA)	Incubate the lysate at room temperature for no longer than 5 minutes.
RNA contamination	Lysate at improper pH, salt concentration, or temperature	• Carefully remove all medium before resuspending cells. • Make sure not to add an excess of Precipitation Buffer (N3) when neutralizing the lysate. • Do not warm the lysate above room temperature during while centrifuging.
	Lysate left on column too long	Once the lysate is loaded onto the column, avoid delays in processing.
	Lysate droplets remained on walls of column at elution	Wash droplets of lysate from the walls of the column with the Wash Buffer (W8).
	RNase A digestion incomplete	• Verify RNase A is added to Resuspension Buffer (R3), and that it was stored at 4°C. • Use recommended volume of buffer R3.

Accessory Products

Additional products

The following products are also available from Thermo Fisher Scientific. For more details on these products, visit **thermofisher.com/lifescience,** or contact **Technical Support** (page 22).

Product	Quantity	Catalog No.
PureLink™ PCR Purification Kit	50 preps	K3100-01
PureLink™ Quick Gel Extraction Kit	50 preps	K2100-12
PureLink™ Nucleic Acid Purification Rack	1 each	K2100-13
PureLink™ HiPure Expi Megaprep	4 preps	K210008 XP
PureLink™ HiPure Expi Gigaprep	2 preps	K210009XP
PureLink™ HiPure Plasmid Filter Midiprep Kits	25 preps 50 preps	K2100-14 K2100-15
PureLink™ HiPure Plasmid Filter Maxiprep Kits	10 preps 25 preps	K2100-16 K2100-17
Expi293™ Expression System Kit	1 kit	A14635
PureLink™ HiPure Plasmid Filter and Precipitator Maxiprep Kit	10 preps 25 preps	K2100-26 K2100-27
Qubit™ dsDNA Assay Kit, Broad-Range	500 assays	Q32853
Qubit™ 2.0 Fluorometer	1 each	Q32866
Luria Broth Base (Miller's LB Broth Base), powder	500 g 2.5 kg	12795-027 12795-084
Ampicillin Sodium Salt, irradiated	200 mg	11593-027

E-Gel™ agarose gels and DNA ladders

E-Gel™ Agarose Gels are bufferless pre-cast agarose gels with a variety of different agarose percentages and well formats designed for fast, convenient electrophoresis of DNA samples.

To find DNA ladders available for sizing DNA, visit **thermofisher.com/lifescience,** or contact **Technical Support** (page 22) for more details on these products.

Technical Support

Obtaining support

For the latest services and support information for all locations, go to **thermofisher.com/lifescience**

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support (**techsupport@lifetech.com**)
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

Safety Data Sheets (SDS)

Safety Data Sheets (SDSs) are available at **thermofisher.com/techresources**

Certificate of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available from **thermofisher.com/lifescience**

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**

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