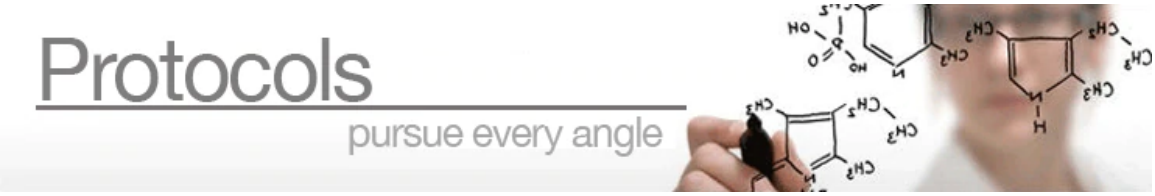
PureLink™ HiPure Plasmid Filter Purification Kits - for Midi and Maxi preparation of Plasmid DNA



Quicklinks

Related Product Information

DNA Purification

Introduction

The PureLink™ HiPure Plasmid Filter Purification Kits allow isolation of high yields of highly pure plasmid DNA. The HiPure Filter Column provides rapid clearing of the bacterial lysate without the need for centrifugation. The lysate Filtration Cartridge is integrated into the DNA Binding Column and combines the steps of clearing the bacterial lysate with binding the DNA directly to the anionexchange resin. The HiPure Filter Column protocol reduces time and effort for plasmid purification. The kits are designed to efficiently isolate plasmid DNA from E. coli in 1.5–2.5 hours using anion-exchange columns without the use of any organic solvents or cesium chloride (CsCl). The isolated plasmid DNA purity is equivalent to two passes through CsCl gradients and has low endotoxin levels.

Kit Formats

The PureLink™ HiPure Plasmid Filter Purification Kits are available in the following formats:

- The PureLink™ HiPure Plasmid Filter Midiprep and Maxiprep Kits allow you to purify plasmid DNA using different starting culture volumes.
- The PureLink™ HiPure Plasmid FP (Filter and Precipitator) Maxiprep Kit includes the PureLink™ HiPure Plasmid Filter Maxiprep Kit and PureLink™ HiPure Precipitator Module, and allows plasmid DNA purification including isopropanol precipitation without centrifugation within one hour.

The HiPure Technology

The HiPure technology is based on anion-exchange chromatography and uses a patented resin composed of small particles with a uniform pore size. The HiPure technology provides high yields of highly pure plasmid DNA with reproducible performance.

The spacer arm with increased length provides improved DNA binding efficiency. The unique patented ion-exchange moiety provides high efficiency separation of DNA from cellular contaminants, including RNA. Filter Columns The HiPure Filter Columns contain the Filtration Cartridge prepackaged in the DNA Binding Column. The column is fitted with anion-exchange resin (see below). This design of the HiPure Filter Column allows sample clarification by filtration and plasmid DNA binding in one combined step.

PureLink™ Column Holder

The Column Holders in the kit allow Midi and Maxi Columns to be supported in an upright position when placed in the mouth of an Erlenmeyer (or similar) flask.

PureLink™ HiPure Precipitator Module

The PureLink™ HiPure Precipitator Module, included with the PureLink™ HiPure Plasmid FP (Filter and Precipitator) Maxiprep Kit (cat. nos. K2100-26 and K2100-27), allows rapid isopropanol precipitation of the eluted DNA without any centrifugation steps. This saves time and reduces the risk of losing the DNA pellet during supernatant removal. To precipitate and desalt the DNA, isopropanol is added to the eluted DNA and then applied to the HiPure Precipitator using a large syringe. After a subsequent washing and drying step, the plasmid DNA is easily eluted from the HiPure Precipitator with TE buffer.

System Overview

The PureLink™ HiPure Plasmid Filter Purification Kits use a patented anion-exchange resin to purify plasmid DNA to a level of purity that is equivalent to two passes through CsCl gradients. The patented resin provides high binding capacity with fast flow rates, high resolution, high yield, and efficient endotoxin removal. E. coli cells are harvested by centrifugation, then resuspended in Resuspension Buffer (R3) with RNase A, and lysed with Lysis Buffer (L7). Precipitation Buffer (N3) is then added to the lysate. The lysate is poured into a pre-packed anion-exchange column fitted with the Filtration Cartridge unit. In one simple combined step, the lysate is clarified and the negatively charged phosphates of the DNA backbone interact with the positive charges on the surface of the anionexchange resin. The temperature, salt concentration, and pH of the solutions are optimized for efficient binding of DNA. A single column wash under moderate salt conditions using Wash Buffer (W8) removes RNA, proteins, carbohydrates and other impurities while the plasmid DNA remains bound to the resin.

The plasmid DNA is eluted under high salt conditions with the Elution Buffer (E4). The eluted DNA is desalted and concentrated by alcohol precipitation or using the PureLink™ HiPure Precipitator Module included with the PureLink™ HiPure Plasmid FP (Filter and Precipitator) Maxiprep Kit or available separately. The entire protocol can be completed in 1.5–2 hours.

Advantages

The PureLink™ HiPure Plasmid Filter Purification Kits offer the following advantages:

- Bacterial lysate clarification by a simple filter column procedure without centrifugation
- No centrifugation required for alcohol precipitation when using the PureLink™ HiPure Precipitator Module
- High-quality purified plasmid DNA suited for mammalian transfections
- High plasmid DNA yields with up to 350 µg for Midipreps and 850 µg for Maxipreps
- Reliable performance of the purified plasmid DNA in a variety of downstream applications

System Specifications

Specifications	Midiprep	Maxiprep
Starting E. coli culture volume	15–25 mL	100–200 mL
Column Binding Capacity**	350 µg	850 µg

Filtration Cartridge Reservoir Capacity	~60 mL	60 mL
Column Reservoir Capacity	~60 mL	60 mL
Elution Volume	5 mL	15 mL
DNA Recovery	90–95%	90–95%
Expected DNA Yield***	100–350 µg	500–850 µg

* Specifications and results are based on high copy number plasmids.
** Binding capacity depends on 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 Plasmid Filter Purification Kits is suitable for a wide variety of downstream applications such as:

- Mammalian transfections
- Automated fluorescent DNA or manual sequencing
- PCR
- Cloning
- *in vitro* transcription
- Restriction digestion.

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Ordering Information

Catalog #	**K210014**
Name	**PureLink™ HiPure Plasmid Filter Midiprep Kit**
Size	25 preps
Price (USD)	313.00
Qty	

Add to cart

Catalog #	**K210015**
Name	**PureLink™ HiPure Plasmid Filter Midiprep Kit**
Size	50 preps
Price (USD)	614.00
Qty	

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Catalog #	**K210016**
Name	**PureLink™ HiPure Plasmid Filter Maxiprep Kit**
Size	10 preps
Price (USD)	266.00
Qty	

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Catalog #	**K210017**
Name	**PureLink™ HiPure Plasmid Filter Maxiprep Kit**
Size	25 preps
Price (USD)	632.00
Qty	

Add to cart

Catalog #	**K210026**
Name	**PureLink™ HiPure Plasmid FP (Filter and Precipitator) Maxiprep Kit**
Size	10 preps
Price (USD)	289.00
Qty	

Add to cart

Catalog #	**K210027**
Name	**PureLink™ HiPure Plasmid FP (Filter and Precipitator) Maxiprep Kit**
Size	25 preps
Price (USD)	688.00
Qty	

Add to cart

Experienced Users Procedure

Introduction

This quick reference section is included for experienced users of the PureLink™ HiPure Plasmid Filter Midiprep, Maxiprep and FP Maxiprep Kits. If you are a first time user of these kits, refer to the detailed protocols in later sections.

Step	Action

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Preparing Cell Lysate

Before Beginning: Apply Equilibration Buffer (EQ1) to Midiprep columns (15 mL) and Maxiprep columns (30 mL). Verify the Resuspension Buffer (R3) contains RNase A, and that no precipitate has formed in the lysis Buffer (L7).

Plasmid Number	Midiprep	Maxiprep
High Copy Number	15–25 mL	100–200 mL
Low Copy Number	25–100 mL	250–500 mL

1. Grow bacterial culture overnight in LB medium. Place appropriate amount of culture in a 50 mL disposable centrifuge tube for cell harvesting (see table above).

2. Harvest cells by centrifugation at 4,000 × g for 10 minutes. Remove all medium. **Note:** If you are using >200 mL culture of high copy number plasmids for the Maxiprep, double the amount of Resuspension Buffer (R3) with RNase A, Lysis Buffer (L7), and precipitation Buffer (N3) for best result

3. Resuspend cell pellet in 10 mL Resuspension Buffer (R3) with RNase A. Gently shake until cell suspension is homogenous.

4. Transfer cell suspension to a new 50 mL centrifuge tube.

5. Add 10 mL Lysis Buffer (L7). Mix until homogenous.

6. Add 10 mL Precipitation Buffer (N3) to the lysate. Mix by inverting the capped tube gently

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Step	Action

Eluting DNA

1. Place a sterile 15 mL (Midi) or 50 mL (Maxi) centrifuge tube (elution tube) under the HiPure filter column.

2. Add Elution Buffer (E4) to the column to elute the DNA. Allow solution to drain by gravity flow.

Midiprep	Maxiprep
5 mL	15 mL

3. The elution tube contains the DNA. Discard the HiPure Filter Column.

Step	Action

Precipitating DNA

Before starting: If you are using the PureLink™ HiPure Plasmid FP Maxiprep kit and you are precipitating the DNA using the PureLink™ HiPure Precipitator Module, refer to the precipitator protocol. To precipitate DNA using centrifugation, follow the steps below.

1. Add isopropanol to the elution tube containing the DNA.

Midiprep	Maxiprep
3.5 mL	10.5 mL

2. Incubate DNA with isopropanol for 2 minutes at room temperature.

3. Centrifuge the tube for at >12,000 × g for 30 minutes at 4°C. Remove and discard the supernatant.

4. Add 70% ethanol to resuspend the pellet in the tube.

Midiprep	Maxiprep
3 mL	5 mL.

5. Add 10 mL Precipitation Buffer (N3) to the lysate. Mix by inverting the capped tube gently

6. Air-dry the pellet for ~10 minutes.

7. Add TE Buffer (TE) to the tube to resuspend the pellet.

Plasmid Number	Midiprep	Maxiprep
High Copy Number	15–25 mL	100–200 mL
Low Copy Number	25–100 mL	250–500 mL

8. **Optional:** Centrifuge the DNA in TE Buffer (TE) for 1 minute at room temperature at high speed to remove any insoluble particles that may be present. Transfer the supernatant containing the DNA into a fresh tube.

9. Store the purified DNA at –20°C or proceed to the downstream application.

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Methods

Before Starting

Review the information in this section before starting. 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.

Caution: ***Some of the buffers in the PureLink™ HiPure Plasmid Filter Purification Kits contain hazardous chemicals. For your protection, always wear a laboratory coat, disposable gloves, and eye protection when handling the buffers.***

Bacterial Cultures

Grow transformed *E. coli* cells overnight in LB (Luria-Bertani) medium with the appropriate antibiotic. The bacteria 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 bacterial culture in transition between exponential phase and stationary phase.

Plasmid Type and Copy Number

The PureLink™ HiPure Plasmid Filter Purification kits allow purification of all types and sizes of plasmid DNA. 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 grown overnight. Typical yieldsfrom low copy number plasmids are highly dependent upon culture conditions and vector/host strain combinations. If you are using a low copy number plasmid, you will need to use a higher volume of bacterial culture. The table below lists the volumes of bacterial culture required for Midiprep and

Maxiprep plasmid DNA purification depending on the plasmid copy number used.

Type of Plasmid	Midiprep	Maxiprep
High copy number plasmid	15–25 mL	100–200 mL
Low copy number plasmid	25–100 mL	250–500 mL

Specific Protocols

The following protocols are provided for purifying plasmid DNA using the various kits discussed on this page:

- Midiprep kit protocol
- Maxiprep kit protocol

Recommendations:

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 kit.
- Make sure that all equipment coming in contact with DNA is sterile, including pipette tips and tubes.
- Use the PureLink™ Nucleic Acid Purification Rack for column purification (see below).
- Perform the recommended wash steps during purification to obtain the best results.
- Use the TE Buffer (TE) provided or 10 mM Tris-HCl, pH 8.5 to resuspend the DNA pellet.

Purification Rack

The PureLink™ Nucleic Acid Purification Rack is designed specifically for use with PureLink™ HiPure Plasmid Filter Midiprep and Maxiprep Kits. The PureLink™ Nucleic Acid Purification Rack consists of the following:

- Column Holder Rack (for processing 12 miniprep, 8 midiprep, and 4 maxiprep columns)
- Collection Tube Rack (capable of accommodating various types and sizes of recovery tubes)
- Large Capacity Waste Tray for collecting waste.

Using the Column Holder

The Column Holders provided in the kit allow columns to be supported in an upright position when placed in the mouth of a flask.

To use the Column Holder, slip the column through the hole in the center of the Column Holder. The column with Column Holder can then be placed in the mouth of a flask.

Buffers

Resuspension Buffer (R3)

Add RNase A to the Resuspension Buffer (R3) according to instructions on the label of the bottle. Mix well. Mark the bottle label to indicate that it contains RNase A (100 µg/ml final concentration). Store the buffer with RNase A at 4°C.

Lysis Buffer (L7)

Check the Lysis Buffer (L7) for precipitates. If present, warm the solution briefly at 37°C to dissolve the precipitate.

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Midiprep Procedure

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Introduction

The PureLink™ HiPure Plasmid DNA Midiprep Kit allows purification of 100–350 µg of high-quality plasmid DNA from 15–25 mL overnight E. coli cultures in ~2 hours when cloning high copy number plasmids.

Before Starting

Before beginning, verify that the Resuspension Buffer (R3) contains RNase A, and no precipitate has formed in the Lysis Buffer (L7).

Materials Needed

- Overnight culture of transformed *E. coli* cells
- Isopropanol
- 70% ethanol
- Sterile, microcentrifuge tubes
- PureLink™ Nucleic Acid Purification Rack
- Tubes or centrifuge bottles for harvesting cells
- Centrifuge and rotor appropriate for harvesting cells
- Appropriate 15-mL centrifuge tubes capable of
- withstanding centrifugation forces >12,000 × g
- Centrifuge capable of centrifuging at >12,000 × g at 47°C
- *Optional*: PureLink™ HiPure Precipitator Module

Components Supplied with the Kit

- Resuspension Buffer (R3) with RNase A
- Lysis Buffer (L7)
- Precipitation Buffer (N3)
- Equilibration Buffer (EQ1)
- Wash Buffer (W8)
- Elution Buffer (E4)
- TE Buffer (TE)
- HiPure Filter Midi Columns
- Column Holder

Note: The protocol for the Midiprep kit of the PureLink™ HiPure Plasmid Filter Purification Kits is designed for purification of both high and low copy number plasmids without the need for adjusting buffer volumes.

Equilibrating the Column

The PureLink™ HiPure Filter Midi Columns are packaged with the Filtration Cartridge pre-inserted into the column housing.

To Equilibrate the column:

1. Use the Column Holder to support a HiPure Filter Midi Column in the mouth of a flask, or place the Midi Column on the PureLink™ Nucleic Acid Purification Rack (see manual supplied with the rack for more details).

2. Apply 15 mL Equilibration Buffer (EQ1) directly to the filtration cartridge in the Midi Column.

3. Allow the solution in the HiPure Filter Midi Column to drain by gravity flow.

Prepare the cell lysate (see below) while the HiPure Filter Midi Column is equilibrating.

Preparing Cell Lysate

1. Harvest bacterial culture cells by centrifugation. For high copy number plasmids, harvest 15–25 mL of an overnight LB culture per sample in a 50-mL disposable tube. For low copy number plasmids, harvest 25–100 mL of an overnight LB culture per sample in a 50-mL disposable tube.

2. Centrifuge the cells at 4,000 × g for 10 minutes to harvest the cells. Remove all medium.

3. Add 10 mL Resuspension Buffer (R3) with RNase A to the cell pellet in the tube and resuspend the cells. Gently shake the tube until cell suspension is homogeneous.

4. Add 10 mL Lysis Buffer (L7). Place the cap on the tube and ensure it is secure. Mix gently by inverting the capped tube until the lysate mixture is thoroughly homogenous. **Do not vortex.**

5. Incubate the lysate at room temperature for 5 minutes. **Do not exceed 5 minutes.**

6. Add 10 mL Precipitation Buffer (N3) and mix immediately by inverting the tube until the mixture is thoroughly homogeneous. Do not vortex.

7. Proceed to Loading Filter Column and Washing DNA

Loading Filter Column and Washing DNA

1. Transfer the precipitated lysate from Step 6 (above), including all the precipitated material into the equilibrated HiPure Filter Midi Column. Let the lysate pass through the filter by gravity flow until the flow stops (10–15 minutes) or becomes very slow (<1 drop per 10 seconds). Discard the flow-through. **Optional:** The final DNA yield may be increased by washing the residual bacterial lysate in the HiPure Filter Midi column with 10 mL Wash Buffer (W8). Again, let the buffer flow through the HiPure Filter Midi Column by gravity flow until the flow stops or dripping becomes very slow.

2. Immediately after the HiPure Filter Midi Column has stopped dripping, remove and discard the inner Filtration Cartridge from the column. **Note:** Do not reuse the Filtration Cartridge. The cartridge is designed for single use only.

3. Wash the Midi column with 20 mL Wash Buffer (W8). Allow the solution in the column to drain by gravity flow. Discard the flow-through.

4. Proceed to Eluting DNA (below)**.**

Eluting DNA

1. Place a sterile 15-mL centrifuge tube (elution tube) under the HiPure Filter Midi column.

2. Add 5 mL Elution Buffer (E4) to the Midi column to elute the DNA. Allow the solution to drain by gravity flow. **Do not** force out any remaining solution. The elution tube contains the purified DNA.

3. Discard the HiPure Filter Midi column.

4. Proceed to Precipitating DNA

Note: For DNA precipitation using the Midiprep Kit, you can use the PureLink™ HiPure Precipitator Module which allows DNA precipitation within 10 minutes without centrifugation, or you can follow the protocol for Precipitating DNA with Isopropanol (below) to perform traditional DNA precipitation using centrifugation. Refer to the manual supplied with the PureLink™ HiPure Precipitator Module for a detailed protocol.

Precipitating DNA with Isopropanol

1. Add 3.5 mL isopropanol to the elution tube containing the DNA (see Eluting DNA). Mix well.

2. Incubate the DNA-isopropanol mixture for 2 minutes at room temperature.

3. Centrifuge the tube at >12,000 × g for 30 minutes at 4°C. Carefully remove and discard the supernatant.

4. Resuspend the DNA pellet in 3 mL 70% ethanol.

5. Centrifuge the tube at >12,000 × g for 5 minutes at 4°C. Carefully remove and discard the supernatant.

6. Air-dry the pellet for ~10 minutes.

7. Resuspend the DNA pellet in 200 µL TE Buffer (TE) for high copy number plasmids. For low copy number plasmids, use 100 µL TE Buffer (TE).

Note: Occasionally, insoluble particles may be present. These particles do not influence the quality of the DNA and can be easily removed. To remove insoluble particles, centrifuge the DNA solution at high speed for 1 minute at room temperature Transfer the supernatant (DNA sample) into a fresh tube.

Storing DNA

Store the purified DNA at –20°C, or proceed to the desired downstream application. **Note:** To avoid repeated freezing and thawing of DNA, store the purified DNA at 4°C for immediate use or aliquot the DNA and store at –20°C for long-term storage.

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Maxiprep Procedure

Introduction

The PureLink™ HiPure Plasmid DNA Maxiprep Kit allows purification of 500–850 µg of high-quality plasmid DNA from 100–200 mL overnight *E. coli* cultures in ~2 hours when cloning high copy number plasmids.

Before Starting

Verify that the Resuspension Buffer (R3) contains RNase A, and no precipitate has formed in the Lysis Buffer (L7).

Materials Needed

- Overnight culture of transformed E. coli cells
- Isopropanol
- 70% ethanol
- Sterile, microcentrifuge tubes
- PureLink™ Nucleic Acid Purification Rack
- Tubes or centrifuge bottles for harvesting cells
- Centrifuge and rotor appropriate for harvesting cells
- 50-mL centrifuge tubes capable of withstanding centrifugation forces >12,000 × g
- Centrifuge capable of centrifuging at >12,000 × g at 4°C

Components Supplied with the Kit

- Resuspension Buffer (R3) with RNase A
- Lysis Buffer (L7)
- Precipitation Buffer (N3)
- Equilibration Buffer (EQ1)
- Wash Buffer (W8)
- Elution Buffer (E4)
- TE Buffer (TE)
- HiPure Filter Maxi Columns
- Column Holder
- PureLink™ HiPure Precipitator Module (supplied with cat. nos. K2100-26 and K2100-27 only)

Note: For Maxipreps of low copy number plasmids from bacterial cultures of >200 mL, use twice the amount of Resuspension Buffer (R3), Lysis Buffer (L7), and Precipitation Buffer (N3) as directed in the protocol.

Order the PureLink™ HiPure BAC Buffer kit for additional buffers if the buffers in the kit are insufficient for using all of the columns when following this protocol.

Equilibrating the Column

The PureLink™ HiPure Filter Maxi Columns are prepackaged with the Filtration cartridge inserted into the column housing.

1. Use the Column Holder to support a HiPure Filter Maxi Column in the mouth a flask, or place the Maxi Column on the PureLink™ Nucleic Acid Purification Rack (see manual supplied with the rack for more details).

2. Apply 30 mL Equilibration Buffer (EQ1) directly into the Filtration Cartridge, which is inserted into the Maxi Column.

3. Allow the solution in the HiPure Filter Maxi Column to drain by gravity flow.

4. Prepare the cell lysate (see below) while the HiPure Filter Maxi Column is equilibrating.

Preparing Cell Lysate

1. For high copy number plasmids, use 100–200 mL of an overnight LB culture per sample. For low copy number plasmids, harvest 250–500 mL of an overnight LB culture per sample.

2. Harvest the cells by centrifuging the overnight LB culture at 4,000 × g for 10 minutes. Remove all medium.

3. Add 10 mL Resuspension Buffer (R3) with RNase A to the pellet and resuspend the cells until homogeneous.

4. Add 10 mL Lysis Buffer (L7). Mix gently by inverting the capped tube until the lysate mixture is thoroughly homogeneous. Do not vortex. Incubate at room temperature for 5 minutes. Note: Do not allow lysis to proceed for more than 5 minutes.

5. Add 10 mL Precipitation Buffer (N3) and mix immediately by inverting the tube until the mixture is thoroughly homogeneous. Do not vortex.

6. Proceed to Loading Filter Column and Washing DNA

Loading Filter Column and Washing DNA

1. Transfer the precipitated lysate from Step 5 in Preparing Cell Lysate including all the precipitated material into the equilibrated HiPure Filter Maxi Column. Let the lysate run through the filter by gravity flow until the flow stops (10–15 minutes) or becomes very slow (<1 drop per 10 seconds). Discard the flow through.

2. Optional: The final DNA yield may be increased by washing the residual bacterial lysate in the HiPure Filter Maxi Column with 10 mL Wash Buffer (W8). Again, let the buffer flow through the HiPure Filter Maxi Column by gravity flow until the flow stops or dripping becomes very slow.

3. Immediately after the HiPure Filter Maxi Column has stopped dripping, remove the inner Filtration Cartridge from the column and discard. Note: Use the HiPure Filtration Cartridge only once. The cartridge is for single use only.

4. Wash the Maxi column with 50 mL of Wash Buffer (W8). Allow the solution in the column to drain by gravity flow. Discard the flow-through.

5. Proceed to Eluting DNA, (below).

Eluting DNA

1. Place a sterile 50-mL centrifuge tube (elution tube) under the HiPure Filter Maxi column.

2. Add 15 mL Elution Buffer (E4) to the Maxi column to elute the DNA. Allow the solution to drain by gravity flow. Do not force out any remaining solution. The elution tube contains the purified DNA.

3. Discard the HiPure Filter Maxi column.

4. Proceed to Precipitating DNA with Isopropanol, (below) or Precipitating DNA Using Precipitator Module

Note: DNA precipitation can be completed using centrifugation or with the PureLink™ HiPure Precipitator Module (included with the FP Maxiprep Kit or purchased as a separate kit). The precipitator module allows DNA precipitation within 10 minutes without any centrifugation steps. Refer to the section below to precipitate DNA with isopropanol by centrifugation. For DNA precipitation using the PureLink™ HiPure Precipitator Module. For a detailed protocol on using the precipitator module, refer to the product insert included with the precipitator.

Precipitating DNA with Isopropanol

1. Add 10.5 mL isopropanol to the DNA to the elution tube. Mix well.

2. Centrifuge the tube at >12,000 × g for 30 minutes at 4°C. Carefully remove and discard the supernatant.

3. Add 5 mL 70% ethanol to resuspend the DNA pellet.

4. Centrifuge the tube at >12,000 × g for 5 minutes at 4°C. Carefully remove and discard the supernatant.

5. Air-dry the pellet for ~10 minutes.

6. Resuspend the DNA pellet in 500 µL TE Buffer (TE). For low copy number plasmids, use 200 µL TE Buffer (TE).

Note: Occasionally, insoluble particles may be present. These particles do not influence the quality of the DNA and can be easily removed. To remove insoluble particles, centrifuge the DNA solution at high speed at room temperature for 1 minute. Transfer the supernatant (DNA sample) into a fresh tube.

Storing DNA

Store the purified DNA at –20°C, or proceed to desired downstream application. **Note:** To avoid repeated freezing and thawing of DNA, store the purified DNA at 4°C for immediate use or aliquot the DNA and store at –20°C for long-term storage.

Follow these guidelines when using the PureLink™ HiPure Precipitator Module (see protocol, below).

- Always remove the precipitator module from the syringe before removing the plunger
- Do not apply excessive pressure while pushing the solution through the precipitator, as too much pressure may detach the precipitator from the syringe.
- Attach the precipitator to the syringe properly using the luer lock mechanism to avoid the detachment of the precipitator during sample processing.
- Always use proper aseptic techniques when working with DNA and use only sterile, DNase-free tips and tubes to prevent DNase contamination.
- When eluting the DNA with TE Buffer (TE), use a higher volume of TE Buffer to increase DNA yield. Use a lower volume of TE Buffer to increase DNA concentration (see Elution Parameters in the precipitator module product insert.
- The TE Buffer (TE) contains 10 mM Tris-HCL, pH 8.0, 0.1mM EDTA). If Tris-HCl or EDTA interferes with downstream applications, sterile water (pH 8.0) can be substituted.

Precipitating DNA Using Precipitator Module

1. Add 10.5 mL isopropanol to the elution tube containing the DNA (see Eluting DNA). Mix well. Incubate for 2 minutes at room temperature.

2. Remove a 30 mL syringe (supplied with the precipitator module) from the package and remove the plunger.

3. Attach the PureLink™ HiPure Precipitator through the luer lock inlet to the 30 mL syringe nozzle.

4. Load the precipitated DNA mixture into the syringe, place the precipitator over a waste container, and insert the plunger into the syringe. Use a slow, constant force to push the plunger to pass the DNA mixture through the precipitator. Discard the flow through.

5. Detach the precipitator from the syringe, remove the plunger, then reattach the precipitator to the syringe. **Note:** To prevent damage to the membrane, do not remove the plunger while the precipitator is still attached to the syringe.

6. To wash the DNA precipitate: Add 3–5 mL 70% ethanol into the syringe. Place the precipitator over a waste container. Insert the plunger into the syringe. Push the plunger to pass the ethanol through the precipitator.

7. Detach the precipitator from the syringe, remove the plunger, then reattach the precipitator to the syringe.

8. To dry the precipitator membrane: Insert the plunger into the syringe and push the plunger to pass air through the precipitator.

9. Repeat Step 8, once.

10. Blot any ethanol droplets on the precipitator nozzle with a paper towel.

11. Detach the precipitator from the 30 mL syringe and discard the 30 mL syringe.

12. Remove a 5 mL syringe (supplied with the precipitator module) from the package and remove the plunger from the syringe. Attach the precipitator to the 5 mL syringe.

13. To elute the plasmid DNA from the precipitator: Add 0.75–1.0 mL TE buffer to the 5 mL syringe. Insert the plunger, and place the precipitator over a clean, sterile microcentrifuge tube. Push the plunger to elute the plasmid DNA into the new tube.

14. **Optional:** Perform a second elution to maximize DNA recovery: Detach the precipitator from the syringe, remove the plunger, and reattach the precipitator to the syringe nozzle. Load the entire volume of eluate from Step 13 into the syringe. Place the precipitator nozzle over a new microcentrifuge tube. Insert and push the plunger to perform the second elution and elute the DNA into the microcentrifuge tube.

15. Store the eluted DNA at –20°C (long-term) or 4°C (short-term), or proceed to downstream application.

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Estimating DNA Yield and Quality

Introduction

Once you have isolated the plasmid DNA, you may determine the quantity and quality of the purified DNA as
described below. DNA Yield Perform DNA quantitation using UV absorbance at 260 nm or Quant-iT™ 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 in a spectrophotometer (using a cuvette with an optical path length of 1 cm) blanked against 10 mM Tris-HCl pH 7.5

2. Calculate the concentration of DNA using the formula: DNA (µg/ml) = A_{260} x 50 × dilution factor For DNA, A_{260} = 1 for a 50 µg/ml solution measured in a cuvette with an optical path length of 1 cm.

Quant-iT™ DNA Assay Kits

The Quant-iT™ DNA Assay Kits provide a rapid, sensitive, and specific method for dsDNA quantitation 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, prediluted standards for standard curve, and a pre-made buffer. The assay is designed for reading in standard fluorescent readers/fluorometers or Qubit® Fluorometer

Estimating DNA Quality

Typically, DNA isolated using the PureLink™ HiPure Plasmid Filter Purification Kit has an A_{260}/A_{280} ratio >1.80 when samples are diluted in Tris-HCl pH 7.5, indicating that the DNA is reasonably clean of proteins that could interfere with downstream applications. Absence of contaminating RNA may be confirmed by agarose gel electrophoresis.

Expected Results

DNA Yield

High copy number plasmid DNA was purified in triplicate from E. coli (TOP10) transformed with pcDNA™ 3.1/His/LacZ using PureLink™ HiPure Plasmid Filter Purification kits as described in this manual. The
plasmid DNA was measured using the Quanti-iT™ Kit. DNA yield information is provided below. **Note:** The plasmid DNA yield depends on plasmid copy number, type, and size, bacterial strain, and growth conditions.

Kit Type	Column Binding Capacity	Starting Culture Volume	DNA Yield
Midiprep	350 µg	15–25 mL	100–350 µg
Maxiprep	850 µg	100–200 mL	500–850 µg

Example Results

Plasmid DNA was isolated as described above. The purified plasmid DNA (100 ng) was analyzed on a 1.2% E-Gel® agarose gel. The 1 Kb Plus DNA Ladder was used as marker (left lane of each gel).

Summary of Expected Results

The summary of expected results using the PureLink™ HiPure Plasmid Filter Purification Kits is listed in the table below.

Kit Type	Midiprep	Maxiprep
Processing Time	~2 hours	~2 hours
Plasmid DNA Yield	100–350 µg	500–850 µg
Endotoxin*	0.1–1.0 EU/µg	0.1–1.0 EU/µg
OD $_{260/280}$	~1.95	~1.98
Sequencing (Capillary)	Successful	Successful
Restriction Enzyme Digestion	Successful	Successful

*** When using pyrogen-free plastic ware and glassware**

TOP

Troubleshooting

Review the information below to troubleshoot your experiments with PureLink™ HiPure Plasmid Filter Purification Kits.

Problem	Cause	Solution
Low plasmid DNA yield	Buffers not stored correctly	• Store Lysis Buffer (L7) and Equilibration Buffer (EQ1) at room temperature. • Store Resuspension Buffer (R3) with added RNase A at 4°C.
	Low copy number plasmid	Increase the volume of starting culture. Carefully remove all medium before resuspending cells. Doubling the volumes of the Resuspension Buffer (R3), Lysis Buffer (L7) and Precipitation Buffer (N3) may help to increase plasmid yield and quality.
	Plasmid DNA pellet over-dried	Do not dry the DNA pellet with a vacuum system. Air-dry the DNA pellet.
	Precipitator membrane damaged resulting in leaks (FP Maxiprep Kit)	• Attach the precipitator to the syringe nozzle using the luer lock mechanism without applying excessive force. • Prior to removing the plunger from the syringe, always remove the precipitator to avoid damaging the membrane. • Do not apply excessive pressure while pushing the solution through the precipitator.
Genomic DNA contamination	Genomic DNA sheared during handling	Gently invert tubes to mix after adding buffers. Do not vortex as it can shear genomic DNA.

Precipitator is clogged (FP Maxiprep Kit)	Too much DNA Applied DNA precipitated with ethanol instead of isopropanol	Only load the eluate from one anion exchange column onto the precipitator. Using eluate from more than one will overload the membrane. Ethanol-precipitated DNA consists of fine particles that may clog the precipitator. Always use isopropanol to precipitate plasmid DNA.
Additional plasmid forms present	Plasmid DNA permanently denatured (band migrating faster than supercoiled DNA)	Incubate the lysate in Lysis Buffer (L7) 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.
	Lysate left on Filter 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 Filter Column with the Wash Buffer (W8).
	RNase A digestion incomplete	• Make sure RNase A is added to Resuspension Buffer (R3). • Use recommended volume of Resuspension Buffer. • Ensure the Resuspension Buffer with RNAse A is stored at 4°C. • If necessary, increase RNase A concentration to 400 µg/ml.
Slow filtration of bacterial lysate from HiPure Filter Columns	Used high culture volumes or overgrown culture	• Reduce volume of culture used. • Remove precipitated cell debris from overgrown cultures by centrifuging the bacterial lysate at 12000 × g for 5 minutes.
Inhibition of downstream reactions	Presence of ethanol in purified DNA	Remove ethanol by air-drying as described in the protocol.

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