



# PureLink™ HiPure Plasmid DNA Purification Kits

**For Mini, Midi, and Maxi preparation of  
Plasmid DNA**

Catalog nos. K2100-02, K2100-03, K2100-04, K2100-05,  
K2100-06, and K2100-07

**Version E**  
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**User Manual**



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# Kit Contents and Storage

## Types of Products

This manual is supplied with the following products:

Product	Quantity	Catalog No.
PureLink™ HiPure Plasmid Miniprep Kit	25 preps	K2100-02
	100 preps	K2100-03
PureLink™ HiPure Plasmid Midiprep Kit	25 preps	K2100-04
	50 preps	K2100-05
PureLink™ HiPure Plasmid Maxiprep Kit	10 preps	K2100-06
	25 preps	K2100-07

## Shipping and Storage

All components of the PureLink™ HiPure Plasmid DNA Purification Kits are shipped at room temperature.

Upon receipt, store all components at room temperature.

## Contents

The components included in the PureLink™ HiPure Plasmid DNA Purification Kits are listed below.

Component	Miniprep		Midiprep		Maxiprep	
	K2100-02	K2100-03	K2100-04	K2100-05	K2100-06	K2100-07
	25 preps	100 preps	25 preps	50 preps	10 preps	25 preps
Resuspension Buffer (R3)	10 ml	50 ml	100 ml	200 ml	100 ml	250 ml
RNase A	100 µl	550 µl	550 µl	1.5 ml	550 µl	1.5 ml
Lysis Buffer (L7)	10 ml	50 ml	100 ml	200 ml	100 ml	250 ml
Precipitation Buffer (N3)	10 ml	40 ml	100 ml	200 ml	100 ml	250 ml
Equilibration Buffer (EQ1)	50 ml	250 ml	250 ml	2 x 250 ml	300 ml	2 x 400 ml
Wash Buffer (W8)	125 ml	500 ml	500 ml	2 x 500 ml	2 x 300 ml	3 x 500 ml
Elution Buffer (E4)	25 ml	90 ml	125 ml	250 ml	200 ml	400 ml
TE Buffer (TE)	15 ml	15 ml	15 ml	30 ml	30 ml	30 ml
HiPure Columns	25 each	100 each	25 each	50 each	10 each	25 each

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## Kit Contents and Storage, Continued

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### Buffer Composition

The composition of buffers included in the PureLink™ HiPure Plasmid Purification 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 Resuspension Buffer (R3)
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

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## Accessory Products

### Additional Products

The following products are also available from Invitrogen. For more details on these products, visit our website at [www.invitrogen.com](http://www.invitrogen.com) or contact **Technical Support** (page 31).

Product	Quantity	Catalog No.
Quant-iT™ DNA Assay Kit, High Sensitivity	1000 assays	Q33120
Quant-iT™ DNA Assay Kit, Broad-Range	1000 assays	Q33130
Qubit™ Fluorometer	1 each	Q32857
PureLink™ Nucleic Acid Purification Rack	1 each	K2100-13
PureLink™ HiPure Plasmid DNA Megaprep	4 preps	K2100-08
PureLink™ HiPure Plasmid DNA Gigaprep	2 preps	K2100-09
PureLink™ HiPure Plasmid Filter Midiprep Kits	25 preps	K2100-14
	50 preps	K2100-15
PureLink™ HiPure Plasmid Filter Maxiprep Kits	10 preps	K2100-16
	25 preps	K2100-17
PureLink™ HiPure BAC Buffer Kit	1 kit	K2100-18
PureLink™ HiPure Precipitator Module	10 preps	K2100-21
	25 preps	K2100-22
ChargeSwitch®-Pro Plasmid Miniprep Kits	50 preps	CS30050
	250 preps	CS30250
Luria Broth Base (Miller's LB Broth Base)®, powder	500 g	12795-027
	2.5 kg	12795-084
Ampicillin Sodium Salt, irradiated	200 mg	11593-027
Carbenicillin, Disodium Salt	5 g	10177-012

### E-Gel® Agarose Gels and DNA Ladders

E-Gel® Agarose Gels are bufferless pre-cast agarose gels designed for fast, convenient electrophoresis of DNA samples. E-Gel® agarose gels are available in different agarose percentages and well formats.

A large variety of DNA ladders is available from Invitrogen for sizing DNA.

For more details on these products, visit our website at [www.invitrogen.com](http://www.invitrogen.com) or contact **Technical Support** (page 31).

# Introduction

## Overview

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### Introduction

The PureLink™ HiPure Plasmid Purification Kits allow isolation of high yields of highly pure plasmid DNA. The kits are designed to efficiently isolate plasmid DNA from *E. coli* in 1.5-2 hours using anion-exchange columns, without the use of any organic solvents or cesium chloride (CsCl). The isolated plasmid DNA is of high purity, equivalent to two passes through CsCl gradients, and contains low endotoxin levels (page 18).

The PureLink™ HiPure Plasmid DNA Purification Kits are available in three formats that allow you to purify plasmid DNA using different starting culture volumes (page 5).

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### The HiPure Technology

The HiPure technology is based on 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

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# Overview, Continued

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## System Overview

The PureLink™ HiPure Plasmid DNA Purification Kits use a patented anion-exchange resin to purify plasmid DNA to a level equivalent to two passes through CsCl gradients. The patented resin provides excellent capacity with fast flow rates, high resolution, high yield, and efficient endotoxin removal.

*E. coli* cells are harvested, resuspended in Resuspension Buffer (R3) with RNase, and then lysed with Lysis Buffer (L7). The Precipitation Buffer (N3) is added to the lysate and the lysate is clarified by centrifugation. The cleared lysate is passed through a pre-packed anion exchange column. The negatively charged phosphates on the backbone of the DNA interact with the positive charges on the surface of the resin. The temperature, salt concentration, and pH of the solutions influence binding. Under moderate salt conditions, plasmid DNA remains bound to the resin while RNA, proteins, carbohydrates and other impurities are washed away with Wash Buffer (W8). The plasmid DNA is eluted under high salt conditions with the Elution Buffer (E4).

The eluted DNA is desalted and concentrated with an alcohol precipitation step. The entire protocol can be completed in 1.5-2 hours.

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## Advantages

The advantages of using PureLink™ HiPure Plasmid DNA Purification Kits are:

- Purification of all types and sizes of plasmid DNA, including BAC, bacmids, and ssM13 DNAs (see page 19)
  - High-quality purified plasmid DNA suited for mammalian transfections
  - High yield of plasmid DNA (next page)
  - Reliable performance of the purified plasmid DNA in a variety of applications (next page)
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## Overview, Continued

### System Specifications

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Specification*	Miniprep	Midiprep	Maxiprep
Starting culture volume	1-3 ml	15-25 ml	100-200 ml
Column Binding Capacity**	30 µg	350 µg	850 µg
Column Reservoir Capacity	2.5 ml	10 ml	60 ml
Elution Volume	0.9 ml	5 ml	15 ml
DNA Recovery	90-95%	90-95%	90-95%
Expected DNA Yield***	≤30 µg	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.

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### Downstream Applications

The purified DNA is ultrapure and suitable for downstream applications, including those requiring the highest purity, such as:

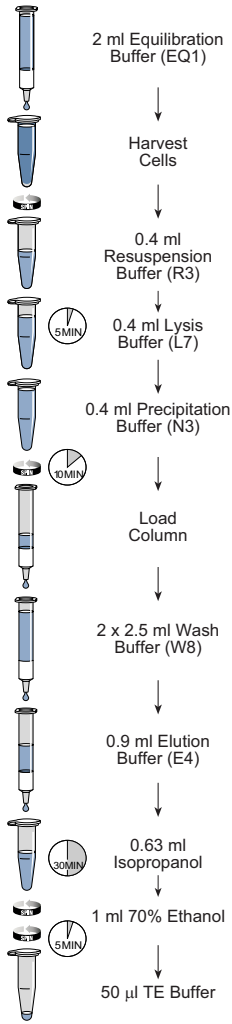
- Transfection of mammalian cells
  - Automated and manual DNA sequencing
  - PCR amplification
  - *In vitro* transcription
  - Bacterial cell transformation
  - Cloning
  - Labeling
-

# Experimental Overview

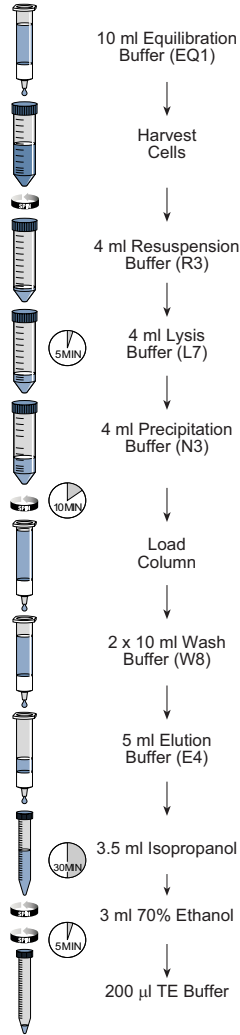
## Introduction

The flow chart for purifying plasmid DNA using the PureLink™ HiPure Plasmid DNA Purification Kits is shown below.

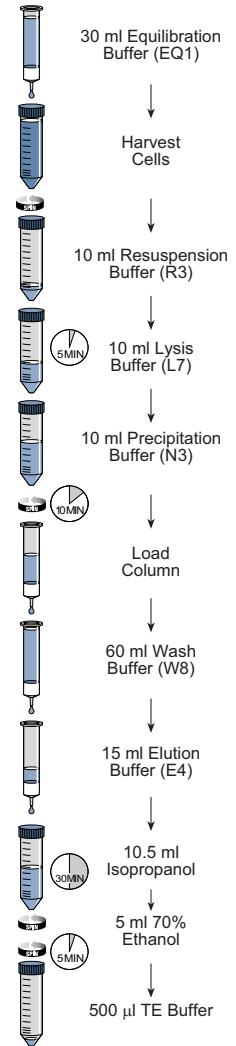
### MiniPrep



### MidiPrep



### MaxiPrep



# Methods

## Before Starting

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### Introduction

Review the information in this section before starting. Guidelines are included for growing the overnight cell culture and for determining the appropriate amounts of starting material based on the plasmid copy number used.

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Some buffers in the PureLink™ HiPure Plasmid DNA Purification Kit contain hazardous chemicals.

Always wear a laboratory coat, disposable gloves, and eye protection when handling the buffers.

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### Bacterial Cultures

Grow transformed *E. coli* cells overnight in LB (Luria-Bertani) medium with the appropriate antibiotic. The bacterial culture should have a cell density of approximately  $10^9$  cells/ml or an optical density of 2.0 at 600 nm ( $OD_{600}$ ). Use a bacterial culture in transition between exponential phase and stationary phase.

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### Plasmid Type and Copy Number

The PureLink™ HiPure Plasmid DNA Purification Kits allow purification of all types and sizes of plasmid DNA, including BAC, bacmids, and ssM13 DNAs (see page 19).

Use a high copy number plasmid to obtain a good yield of plasmid DNA. High copy-number plasmids typically yield 2-6  $\mu$ g DNA/ml LB culture grown overnight. 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 cell culture, as directed in the protocol.

The table below lists the volumes of cell culture recommended for Miniprep, Midiprep, and Maxiprep plasmid DNA purification depending on the plasmid copy number.

Plasmid Copy Number	Miniprep	Midiprep	Maxiprep
High-copy number plasmid	1-3 ml	15-25 ml	100-200 ml
Low-copy number plasmid	10-15 ml	25-100 ml	250-500 ml

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## Before Starting, Continued

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### Note

If you are performing Maxipreps using low copy number plasmids with bacterial cultures of >200 ml, you need to use twice the amount of Resuspension Buffer (R3), Lysis Buffer (L7), and Precipitation Buffer (N3) as directed in the Maxiprep protocol.

The amount of buffers provided in the Maxiprep kit might not be sufficient to utilize all of the columns provided in the kit. Additional buffers for the Maxiprep kit may be obtained by ordering the PureLink™ HiPure BAC Buffer kit from Invitrogen (page vi).

The Midiprep protocol is designed for purification from high and low copy number plasmids without the need for adjusting buffer volumes.

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### Specific Protocols

Specific protocols for plasmid DNA purification using the various kits are described in this manual as shown in the table below.

Protocol	Page no.
Purifying plasmid DNA using:	
Miniprep kit	8
Midiprep kit	11
Maxiprep kit	14
Purifying BAC DNA	19
Purifying Bacmid DNA	22
Purifying Cosmid DNA	24
Purifying ssM13 DNA	26

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## Before Starting, Continued

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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.
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### Purification Rack

The PureLink™ Nucleic Acid Purification Rack (available from Invitrogen, page vi) is designed specifically for use with PureLink™ HiPure Plasmid DNA Miniprep, Midiprep, and Maxiprep Kits. The PureLink™ Nucleic Acid Purification Rack consists of a Column Holder Rack (for processing 12 miniprep, 8 midiprep, and 4 maxiprep columns), a Collection Tube Rack (capable of accommodating various types and sizes of recovery tubes), and a large capacity Waste Tray for collecting waste.

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### Buffer

#### 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 RNase A is added. Store the buffer with RNase 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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# Miniprep Procedure

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## Introduction

The PureLink™ HiPure Plasmid DNA Miniprep Kit allows purification of up to 30 µg of high quality plasmid DNA from high copy number plasmids using 1-3 ml overnight *E. coli* cultures in ~1 hour.

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## Before Starting

Before beginning, verify that RNase A is added to the Resuspension Buffer (R3) and that no precipitate has formed in the Lysis Buffer (L7, page 7).

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## Materials Needed

- Overnight culture of transformed *E. coli* cells (page 5)
  - Isopropanol
  - 70% ethanol
  - Sterile, microcentrifuge tubes
  - PureLink™ Nucleic Acid Purification Rack (page vi)
  - Microcentrifuge capable of centrifuging at >12,000 x g
- 

## Components Supplied with the Kit

- Resuspension Buffer (R3) with RNase A (page 7)
  - Lysis Buffer (L7)
  - Precipitation Buffer (N3)
  - Equilibration Buffer (EQ1)
  - Wash Buffer (W8)
  - Elution Buffer (E4)
  - TE Buffer (TE)
  - PureLink™ HiPure Mini Columns
- 

## Equilibrating the Column

Place the PureLink™ HiPure Mini column on the PureLink™ Nucleic Acid Purification Rack (see the manual supplied with the rack for more details). Apply 2 ml Equilibration Buffer (EQ1) to the column. Allow the solution in the column to drain by gravity flow. Proceed to lysate preparation (next page) while the column is equilibrating.

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# Miniprep Procedure, Continued

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## Preparing Cell Lysate

1. For **high copy number plasmids**, use 1-3 ml of an overnight LB culture per sample in a microcentrifuge tube.  
**Note:** When using 2-3 ml of culture, pellet 1-1.5 ml culture *twice* in the *same* microcentrifuge tube. If you are using >5 ml of culture volume of high copy plasmids, add twice the amount of Resuspension Buffer (R3) with RNase A, Lysis Buffer (L7), and Precipitation Buffer (N3) as directed in Steps 3, 4, and 5, below for best results  
For **low copy number plasmids**, use 10-15 ml of an overnight LB culture per sample in a 15-ml disposable tube.
2. Harvest the cells by centrifuging the overnight LB-culture at 4,000 × g for 5-10 minutes. Remove all medium.
3. Add 0.4 ml Resuspension Buffer (R3) with RNase A to the pellet and resuspend cells until homogeneous.  
**Note:** If cells were resuspended in a 15-ml disposable tube, then transfer the cells in a microcentrifuge tube.
4. Add 0.4 ml Lysis Buffer (L7). Mix gently by inverting the capped tube five times. **Do not vortex.** Incubate at room temperature for 5 minutes.  
**Note:** Do not allow lysis to proceed for more than 5 minutes.
5. Add 0.4 ml Precipitation Buffer (N3) and mix immediately by inverting the tube until the mixture is homogeneous. **Do not vortex.**
6. Centrifuge the lysate at >12,000 × g for 10 minutes at room temperature.  
**Note:** If the pellet does not adhere to the bottom of the tube, incubate the tube at room temperature for 5 minutes to allow the separation of lysate and gelatinous pellet. Pipette the clear lysate into another sterile tube and centrifuge at >15,000 × g for 5 minutes at room temperature to remove any remaining cellular debris.
7. Proceed to **Binding and Washing DNA** (next page).

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# Miniprep Procedure, Continued

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## Binding and Washing DNA

1. Load the supernatant from Step 6 (previous page) onto the equilibrated column. Allow the solution in the column to drain by gravity flow.
  2. Wash the column **twice** with 2.5 ml Wash Buffer (W8). Allow the solution in the column to drain by gravity flow after each wash. Discard the flow-through.
  3. Proceed to **Eluting and Precipitating DNA**, below.
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## Eluting and Precipitating DNA

1. Place a sterile microcentrifuge tube (elution tube) under the column.
  2. Add 0.9 ml Elution Buffer (E4) to the 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.* Discard the column.
  3. Add 0.63 ml isopropanol to the elution tube. Mix well.
  4. Centrifuge the elution tube at  $>12,000 \times g$  for 30 minutes at  $4^{\circ}\text{C}$ . Carefully remove and discard the supernatant.
  5. Resuspend the DNA pellet in 1 ml 70% ethanol.
  6. Centrifuge at  $>12,000 \times g$  for 5 minutes at  $4^{\circ}\text{C}$ . Carefully remove and discard the supernatant.
  7. Air-dry the pellet for 10 minutes.
  8. Resuspend the DNA pellet in 50  $\mu\text{l}$  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.
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## Storing DNA

- Store the purified DNA at  $-20^{\circ}\text{C}$  or use DNA for the desired downstream application.
  - To avoid repeated freezing and thawing of DNA, store the purified DNA at  $4^{\circ}\text{C}$  for immediate use or aliquot the DNA and store at  $-20^{\circ}\text{C}$  for long-term storage.
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# Midiprep Procedure

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## Introduction

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

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## Before Starting

Before beginning, verify that RNase A is added to the Resuspension Buffer (R3) and that no precipitate has formed in the Lysis Buffer (L7, page 7).

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## Materials Needed

- Overnight culture of transformed *E. coli* cells (page 5)
  - Isopropanol
  - 70% ethanol
  - Sterile, microcentrifuge tubes
  - PureLink™ Nucleic Acid Purification Rack (page vi)
  - Tubes or buckets of appropriate size for harvesting cells
  - Centrifuge and rotor appropriate for harvesting cells
  - Appropriate centrifuge tubes capable of withstanding centrifugation forces > 15,000 x g
  - Centrifuge capable of centrifuging at >15,000 x g at 4°C
  - *Optional:* PureLink™ HiPure Precipitator Module (page vi)
- 

## Components Supplied with the Kit

- Resuspension Buffer (R3) with RNase A (page 7)
  - Lysis Buffer (L7)
  - Precipitation Buffer (N3)
  - Equilibration Buffer (EQ1)
  - Wash Buffer (W8)
  - Elution Buffer (E4)
  - TE Buffer (TE)
  - PureLink™ HiPure Midi Columns
- 

## Equilibrating the Column

Place the PureLink™ HiPure Midi column on the PureLink™ Nucleic Acid Purification Rack (see the manual supplied with the rack for more details). Apply 10 ml Equilibration Buffer (EQ1) to the column. Allow the solution in the column to drain by gravity flow. Proceed to lysate preparation (next page), while the column is equilibrating.

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# Midiprep Procedure, Continued

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## Preparing Cell Lysate

1. For **high copy number plasmids**, use 15-25 ml of an overnight LB culture per sample in a disposable 50-ml disposable tube.

**Note:** If you are using >25 ml of culture volume of high copy plasmids, add twice the amount of Resuspension Buffer (R3) with RNase A, Lysis Buffer (L7), and Precipitation Buffer (N3) as directed in Steps 3, 4, and 5, below for best results.

For **low copy number plasmids**, use 25-100 ml of an overnight LB culture per sample in a 50-ml disposable tube.

2. Harvest the cells by centrifuging the overnight LB-culture at 4,000 x g for 10 minutes in a bucket. Remove all medium.
3. Add 4 ml Resuspension Buffer (R3) with RNase A to the cell pellet and resuspend the cells until homogeneous. Transfer the cell suspension to a 15-ml centrifuge tube.
4. Add 4 ml Lysis Buffer (L7). Mix gently by inverting the capped tube five times. **Do not vortex.** Incubate at room temperature for 5 minutes.

**Note:** Do not allow lysis to proceed for more than 5 minutes.

5. Add 4 ml Precipitation Buffer (N3) and mix immediately by inverting the capped tube until the mixture is homogeneous. **Do not vortex.**
6. Centrifuge the mixture at >12,000 x g for 10 minutes at room temperature.

**Note:** If the pellet does not adhere to the bottom of the tube, incubate the tube at room temperature for 5 minutes to allow the separation of the lysate and gelatinous pellet. Pipette the clear lysate into another, sterile tube and centrifuge at >15,000 x g at room temperature for 5 minutes to remove any remaining cellular debris.

7. Proceed to **Binding and Washing DNA**, below.
- 

## Binding and Washing DNA

1. Load the supernatant from Step 6 (above) onto the equilibrated column. Allow the solution in the column to drain by gravity flow.
  2. Wash the column **twice** with 10 ml Wash Buffer (W8). Allow the solution in the column to drain by gravity flow after each wash. Discard the flow-through.
  3. Proceed to **Eluting and Precipitating DNA** (next page).
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# Midiprep Procedure, Continued

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## Note

For DNA precipitation, you can use the PureLink™ HiPure Precipitator Module (page vi) which allows DNA precipitation within 10 minutes without any centrifugation steps, or you can follow the protocol below to perform traditional DNA precipitation using centrifugation.

Refer to the manual supplied with the PureLink™ HiPure Precipitator Module for a detailed protocol.

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## Eluting and Precipitating DNA

1. Place a sterile 15-ml centrifuge tube (elution tube) under the column.
2. Add 5 ml Elution Buffer (E4) on the 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.* Discard the column.

3. Add 3.5 ml isopropanol to the elution tube. Mix well.  
**Note:** Proceed to the protocol described in the PureLink™ HiPure Precipitator manual after this step, if you are using the precipitator module.
4. Centrifuge the tube at  $>15,000 \times g$  for 30 minutes at 4°C. Carefully remove and discard the supernatant.
5. Resuspend the pellet in 3 ml 70% ethanol.
6. Centrifuge the tube at  $>15,000 \times g$  for 5 minutes at 4°C. Carefully remove and discard the supernatant.
7. Air-dry the pellet for 10 minutes.
8. Resuspend the DNA pellet in 200  $\mu$ l TE Buffer (TE). For low copy number plasmids, use 100  $\mu$ l of TE Buffer.

**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.

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## Storing DNA

- Store the purified DNA at -20°C or use DNA for the desired downstream application.
  - 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

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## Introduction

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

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## Before Starting

Before beginning, verify that RNase A has been added to the Resuspension Buffer (R3) and that no precipitate has formed in the Lysis Buffer (L7, page 7).

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## Materials Needed

- Overnight culture of transformed *E. coli* cells (page 5)
  - Isopropanol
  - 70% ethanol
  - Sterile, microcentrifuge tubes
  - PureLink™ Nucleic Acid Purification Rack (page vi)
  - Tubes or buckets of appropriate size for harvesting cells
  - Centrifuge and rotor appropriate for harvesting cells
  - Appropriate centrifuge tubes capable to withstand centrifugation forces >15,000 x g
  - Centrifuge capable of centrifuging at >15,000 x g at 4°C
  - *Optional:* PureLink™ HiPure Precipitator Module (page vi)
- 

## Components Supplied with the Kit

- Resuspension Buffer (R3) with RNase A (page 7)
  - Lysis Buffer (L7)
  - Precipitation Buffer (N3)
  - Equilibration Buffer (EQ1)
  - Wash Buffer (W8)
  - Elution Buffer (E4)
  - TE Buffer (TE)
  - PureLink™ HiPure Maxi Columns
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## Equilibrating the Column

Place the PureLink™ HiPure Maxi column on the PureLink™ Nucleic Acid Purification Rack (see the manual supplied with the rack for more details). Apply 30 ml Equilibration Buffer (EQ1) to the column. Allow the solution in the column to drain by gravity flow. Proceed to lysate preparation (next page) while the column is equilibrating.

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# Maxiprep Procedure, Continued

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## 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**, use 250-500 ml of an overnight LB culture per sample.  
**Note:** For culture volumes >200 ml, add twice the amount of Resuspension Buffer (R3) with RNase A, Lysis Buffer (L7), and Precipitation Buffer (N3) as directed in Steps 3, 4, and 5, below.
  2. Harvest the cells by centrifuging the overnight LB-culture at 4,000 x g for 10 minutes in a bucket. Remove all medium.
  3. Add 10 ml Resuspension Buffer (R3) with RNase A to the pellet and resuspend the cells until homogeneous. Transfer cell suspension to a 50-ml centrifuge tube.
  4. Add 10 ml Lysis Buffer (L7). Mix gently by inverting the capped tube five times. **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 homogeneous. **Do not vortex.**
  6. Centrifuge the mixture at >12,000 x g for 10 minutes at room temperature.  
**Note:** If the pellet does not adhere to the bottom of the tube, incubate the tube at room temperature for 5 minutes to allow the separation of the lysate and gelatinous pellet. Pipette the clear lysate into another tube and centrifuge at >15,000 x g for 5 minutes at room temperature to remove any remaining cellular debris.
  7. Proceed to **Binding and Washing DNA**, below.
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## Binding and Washing DNA

1. Load the supernatant from Step 6 (above) onto the equilibrated column. Allow the solution in the column to drain by gravity flow.
  2. Wash the column with 60 ml Wash Buffer (W8). Allow the solution in the column to drain by gravity flow. Discard the flow-through.
  3. Proceed to **Eluting and Precipitating DNA** (next page).
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*Continued on next page*

## Maxiprep Procedure, Continued

---



### Note

For DNA precipitation, you can use the PureLink™ HiPure Precipitator Module (page vi) which allows DNA precipitation within 10 minutes without any centrifugation steps, or you can follow the protocol below to perform traditional DNA precipitation using centrifugation.

Refer to the manual supplied with the PureLink™ HiPure Precipitator Module for a detailed protocol.

---

### Eluting and Precipitating DNA

1. Place a sterile 30-ml centrifuge tube (elution tube) under the column.
2. Add 15 ml Elution Buffer (E4) to the 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.* Discard the column.

3. Add 10.5 ml isopropanol to the elution tube. Mix well.  
**Note:** Proceed to the protocol described in the PureLink™ HiPure Precipitator manual after this step, if you are using the precipitator.
4. Centrifuge the elution tube at  $>15,000 \times g$  for 30 minutes at 4°C. Carefully remove and discard the supernatant.
5. Resuspend the DNA pellet in 5 ml 70% ethanol.
6. Centrifuge the elution tube at  $>15,000 \times g$  for 5 minutes at 4°C. Carefully remove and discard the supernatant.
7. Air-dry the pellet for 10 minutes.
8. Resuspend the DNA pellet in 500  $\mu$ l TE Buffer (TE). For low copy number plasmids, use 200  $\mu$ l 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 use DNA for the desired downstream application.
  - 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.
-

# Estimating DNA Yield and Quality

---

## Introduction

Once you have isolated 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:  
$$\text{DNA } (\mu\text{g/ml}) = A_{260} \times 50 \times \text{dilution factor}$$

For DNA,  $A_{260} = 1$  for a 50  $\mu\text{g/ml}$  solution measured in a cuvette with an optical path length of 1 cm.

### Quant-iT™ DNA Assay Kits

The Quant-iT™ DNA Assay Kits (page vi) 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, pre-diluted standards for standard curve, and a pre-made buffer. The assay is designed for reading in standard fluorescent readers/fluorometer or Qubit™ Fluorometer.

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## Estimating DNA Quality

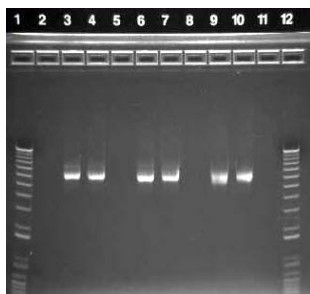
Typically, DNA isolated using the PureLink™ HiPure Plasmid Purification Kit has an  $A_{260}/A_{280}$  ratio  $>1.80$  when samples are diluted in Tris-HCl pH 7.5, indicating that the DNA is reasonably clean of proteins that could interfere with downstream applications. Absence of contaminating RNA may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

---

## Expected Results

### Results

Plasmid DNA was isolated in duplicates from *E. coli* (TOP10) transformed with pcDNA™ 3.1/His/LacZ using the PureLink™ HiPure Plasmid DNA Purification Kits as described in this manual. The purified plasmid DNA was analyzed for yield, endotoxin levels, OD<sub>260/280</sub> ratio, sequencing, restriction enzyme digestion and gel electrophoresis (100 ng) on a 0.8% E-Gel® agarose gel (see below).



Lanes 1, 12: TrackIt™ 1 Kb Plus DNA Ladder  
Lanes 2, 5, 8, 11: Blank  
Lanes 3, 4: Miniprep (3 ml culture)  
Lanes 6, 8: Midiprep (25 ml culture)  
Lanes 9, 10: Maxiprep (100 ml culture)

### Summary of Expected Results

The summary of results using the PureLink™ HiPure Plasmid DNA 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:	Miniprep	Midiprep	Maxiprep
Processing Time	~1 hour	~2 hours	~2 hours
Plasmid DNA Yield*	≤30 µg	100-350 µg	500-850 µg
Column Binding Capacity	30 µg	350 µg	850 µg
Endotoxin	0.1-1 EU/µg	0.1-1 EU/µg	0.1-1.5 EU/µg
OD <sub>260/280</sub>	~1.87	~1.95	~1.98
Sequencing (Capillary)	Successful	Successful	Successful
Restriction Enzyme Digestion	Successful	Successful	Successful

\* As determined by using Quant-iT™ Kit or by measuring UV absorbance at 260 nm



# Appendix

## Procedure for BAC DNA

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### Introduction

The PureLink™ HiPure Plasmid DNA Purification Kits allow you to purify high quality BAC (bacterial artificial chromosome) DNA from *E. coli* cultures.

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### Note

Due to changes in the volumes of buffers used in this protocol, the volume amounts of buffers provided in the PureLink™ HiPure Plasmid DNA Purification Kits may not be sufficient to utilize all of the columns provided in the kit. To obtain additional amounts of buffers order the PureLink™ HiPure BAC Buffer Kit from Invitrogen (page vi).

---

### Before Starting

- Prepare a 20-h culture of BAC containing bacteria in 2X YT medium and appropriate antibiotic. The absorbance at 600 nm of the final culture should be  $5.0 \pm 0.5$ .
  - Increase the NaCl concentration in the Wash Buffer (W8) to 0.9 M NaCl by adding 0.58 g NaCl per 100 ml Wash Buffer (W8). Conductivity should be 72 mS. This increase in salt concentration reduces the RNA contamination in the BAC preparation.
  - Warm an aliquot of Elution Buffer (E4) to 50°C.
  - Add 20 mg/ml RNase A to Resuspension Buffer (R3) to a final concentration of **400 µg/ml**.
  - Verify that no precipitate has formed in the Lysis Buffer (L7, page 7).
- 

### Equilibrating the Column

Place the PureLink™ HiPure column on the PureLink™ Nucleic Acid Purification Rack (see the manual supplied with the rack for more details). Apply Equilibration Buffer (EQ1) to the column. Allow the solution in the column to drain by gravity flow.

	Miniprep	Midiprep	Maxiprep
Volume of EQ1	2 ml	10 ml	30 ml

---

*Continued on next page*

## Procedure for BAC DNA, Continued

---

### Preparing Cell Lysate

1. Harvest bacterial cells by centrifuging at 9,000 x g for 15 minutes. Remove all medium.

	Miniprep	Midiprep	Maxiprep
Culture Volume	10-25 ml	100 ml	200-500 ml

2. Add Resuspension Buffer (R3) containing RNase A to the pellet and resuspend the cells until homogeneous. Transfer cell suspension to a centrifuge tube.

	Miniprep	Midiprep	Maxiprep
Volume of R3	2 ml	8 ml	40 ml

3. Add Lysis Buffer (L7). Mix gently by inverting the capped tube five times. **Do not vortex.** Incubate at room temperature for 5 minutes.

	Miniprep	Midiprep	Maxiprep
Volume of L7	2 ml	8 ml	40 ml

4. Add Precipitation Buffer (N3) and mix immediately by inverting the capped tube until the mixture is homogeneous. **Do not vortex.**

	Miniprep	Midiprep	Maxiprep
Volume of N3	2 ml	8 ml	40 ml

5. Centrifuge the mixture at >15,000 x g at room temperature for 10 minutes.

**Note:** If the pellet does not adhere to the bottom of the tube, incubate the tube at room temperature for 5 minutes to allow the separation of the lysate and gelatinous pellet. Pipette the clear lysate into a sterile tube and centrifuge at >15,000 x g for 5 minutes at room temperature to remove any remaining cellular debris.

---

### Binding and Washing DNA

1. Load the supernatant from Step 5 (above) onto the equilibrated column. Allow the solution in the column to drain by gravity flow.
2. Wash the column once with Wash Buffer (W8). Allow the solution in the column to drain by gravity flow. Discard the flow-through.

	Miniprep	Midiprep	Maxiprep
Volume of W8	2.5 ml	10 ml	60 ml

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*Continued on next page*

# Procedure for BAC DNA, Continued

---

## Eluting and Precipitating DNA

1. Place a sterile centrifuge tube (elution tube) under the column.
2. Add Elution Buffer (E4) **warmed to 50°C** onto the column to elute DNA. Allow the solution to drain by gravity flow. Do not force out any remaining solution. *The elution tube contains the purified DNA.* Discard the column.

	Miniprep	Midiprep	Maxiprep
Volume of E4	0.9 ml	5 ml	15 ml

3. Add 0.7 volumes of isopropanol to the elution tube. Mix well.

	Miniprep	Midiprep	Maxiprep
Volume of isopropanol	0.63 ml	3.5 ml	10.5 ml

4. Centrifuge the mixture at  $>15,000 \times g$  for 30 minutes at 4°C. Carefully remove and discard the supernatant.
5. Resuspend the DNA pellet in 70% ethanol.

	Miniprep	Midiprep	Maxiprep
Volume of 70% ethanol	1 ml	3 ml	5 ml

6. Centrifuge at  $>15,000 \times g$  for 5 minutes at 4°C. Carefully remove and discard the supernatant.
7. Air-dry the pellet for 10 minutes.
8. Resuspend the DNA pellet in TE Buffer (TE).

	Miniprep	Midiprep	Maxiprep
Volume of TE	10 $\mu$ l	50-100 $\mu$ l	200-400 $\mu$ l

**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.

---

## Expected Results

The above procedure allows purification of a ~100 kb BAC molecule with yields of approximately 40  $\mu$ g DNA per 100 ml culture.

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# Procedure for Bacmid DNA

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## Introduction

The PureLink™ HiPure Plasmid DNA Miniprep Kit allows you to purify high quality Bacmid DNA from *E. coli* (DH10Bac™). The isolated bacmid DNA is suitable for use in insect cell transfections.

---

## Before Starting

- Inoculate a single white bacterial colony into 2 ml LB medium with appropriate antibiotics. Incubate the culture at 37°C in a shaking water bath at 250 rpm for a minimum of 1 h to overnight.
  - Verify that RNase A is added to the Resuspension Buffer (R3) and that the Lysis Buffer (L7) contains no precipitates.
- 

## Equilibrating the Column

Place the PureLink™ HiPure Mini column on the PureLink™ Nucleic Acid Purification Rack (see the manual supplied with the rack for more details). Apply 2 ml Equilibration Buffer (EQ1) to the column. Allow the solution in the column to drain by gravity flow.

---

## Preparing Cell Lysate

1. Harvest 1.5 ml bacterial cells by centrifuging at 9,000 x g for 15 minutes. Remove all medium.
2. Add 0.4 ml Resuspension Buffer (R3) containing RNase A to the pellet and resuspend the cells until homogeneous. Transfer cell suspension to a centrifuge tube.
3. Add 0.4 ml Lysis Buffer (L7). Mix gently by inverting the capped tube five times. **Do not vortex.** Incubate at room temperature for 5 minutes.
4. Add 0.4 ml Precipitation Buffer (N3) and mix immediately by inverting the capped tube until the mixture is homogeneous. **Do not vortex.**
5. Centrifuge the mixture at >15,000 x g at room temperature for 10 minutes.

**Note:** If the pellet does not adhere to the bottom of the tube, incubate the tube at room temperature for 5 minutes to allow the separation of the lysate and gelatinous pellet. Pipette the clear lysate into a sterile tube and centrifuge at >15,000 x g for 5 minutes at room temperature to remove any remaining cellular debris.

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*Continued on next page*

# Procedure for Bacmid DNA, Continued

---

## Binding and Washing DNA

1. Load the supernatant from Step 5 (previous page) onto the equilibrated column. Allow the solution in the column to drain by gravity flow.
  2. Wash the column **twice** with 2.5 ml Wash Buffer (W8). Allow the solution in the column to drain by gravity flow after each wash. Discard the flow-through.
- 

## Eluting and Precipitating DNA

1. Place a sterile centrifuge tube (elution tube) under the column.
  2. Add 0.9 ml Elution Buffer (E4) to the column to elute DNA. Allow the solution to drain by gravity flow. Do not force out any remaining solution.  
*The elution tube contains the purified DNA. Discard the column.*
  3. Add 0.63 ml isopropanol to the elution tube. Mix and place on ice for 10 minutes.
  4. Centrifuge the mixture at  $>15,000 \times g$  at  $4^{\circ}\text{C}$  for 20 minutes. Carefully remove and discard the supernatant.
  5. Resuspend the DNA pellet in 1 ml 70% ethanol.
  6. Centrifuge at  $>15,000 \times g$  at  $4^{\circ}\text{C}$  for 5 minutes. Carefully remove and discard the supernatant.
  7. Air-dry the pellet for 10 minutes.
  8. Resuspend the DNA pellet in 40  $\mu\text{l}$  TE Buffer (TE). Allow pellet to dissolve for at least 10 minutes on ice. To avoid shearing the DNA, pipette only 1-2 times to resuspend.
  9. Store the bacmid DNA at  $-20^{\circ}\text{C}$  and avoid repeated freezing and thawing.
-

# Procedure for Cosmid DNA

---

## Introduction

The PureLink™ HiPure Plasmid DNA Miniprep Kit allows you to purify high quality cosmid DNA from *E. coli*.

---

## Before Starting

- Inoculate a bacterial culture containing your cosmid construct in LB medium with the appropriate selective antibiotic and grow the bacteria for 16 h (or overnight) on a 225 rpm shaking incubator.
  - Add 20 mg/ml RNase A to Resuspension Buffer (R3) to a final concentration of **100 µg/ml**.
  - Verify that no precipitate has formed in the Lysis Buffer (L7, page 7).
- 

## Equilibrating the Column

Place the PureLink™ HiPure Mini column on the PureLink™ Nucleic Acid Purification Rack (see the manual supplied with the rack for more details). Apply 2 ml Equilibration Buffer (EQ1) to the column. Allow the solution in the column to drain by gravity flow.

---

## Preparing Cell Lysate

1. Harvest 3 ml cells by centrifuging at 9,000 x g for 15 minutes. **Thoroughly** remove all medium.
2. Add 0.4 ml Resuspension Buffer (R3) containing **100 µg/ml** RNase A to the pellet and resuspend the cells until homogeneous. Transfer cell suspension to a centrifuge tube.
3. Add 0.4 ml Lysis Buffer (L7). Mix gently by inverting the capped tube five times. **Do not vortex**. Incubate at room temperature for 5 minutes.
4. Add 0.4 ml Precipitation Buffer (N3) and mix immediately by inverting the capped tube until the mixture is homogeneous. **Do not vortex**.
5. Centrifuge the mixture at >12,000 x g at room temperature for 10 minutes.

**Note:** If the pellet does not adhere to the bottom of the tube, incubate the tube at room temperature for 5 minutes to allow the separation of the lysate and gelatinous pellet. Pipette the clear lysate into a sterile tube and centrifuge at >15,000 x g at room temperature for 5 minutes to remove any remaining cellular debris.

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*Continued on next page*

# Procedure for Cosmid DNA, Continued

---

## Binding and Washing DNA

1. **Pipette** the supernatant from Step 5 (previous page) onto the equilibrated column. Allow the solution in the column to drain by gravity flow.
  2. Wash the column **twice** with 2.5 ml Wash Buffer (W8). Allow the solution in the column to drain by gravity flow after each wash. Discard the flow-through.
- 

## Eluting and Precipitating DNA

1. Place a sterile centrifuge tube (elution tube) under the column.
  2. Add 0.9 ml Elution Buffer (E4) to the column to elute DNA. Allow the solution to drain by gravity flow. Do not force out any remaining solution.  
*The elution tube contains the purified DNA.* Discard the column.
  3. Add 0.63 ml of isopropanol to the elution tube. Mix and place on ice for 10 minutes.
  4. Centrifuge the mixture at  $>15,000 \times g$  at  $4^{\circ}\text{C}$  for 20 minutes. Carefully remove and discard the supernatant.  
**Note:** It is highly recommended to use a swinging rotor.
  5. Resuspend the DNA pellet in 1 ml 70% ethanol.
  6. Centrifuge at  $>12,000 \times g$  at  $4^{\circ}\text{C}$  for 5 minutes. Carefully remove and discard the supernatant.
  7. Air-dry the pellet for 10 minutes.
  8. Resuspend the DNA pellet in 50  $\mu\text{l}$  TE Buffer (TE).  
**Note:** If a fixed-angle rotor was used for the centrifugation, the pellet will be spread over the tube walls. Make sure to wash off the tube walls when resuspending the pellet.
- 

## Expected Results

This procedure allows purification of a ~45 kb cosmid DNA with yields of ~4  $\mu\text{g}$  DNA per 3 ml culture.

---

# Procedure for ssM13 DNA

---

## Introduction

The PureLink™ HiPure Plasmid DNA Purification Kits allow you to purify high quality ssM13 (single strand M13) DNA from bacteria.

---



## Note

When using the PureLink™ HiPure Plasmid DNA Purification Kits for this procedure, note that the number of reactions may vary from that stated on page 3 because of changes in the volumes used with the kit-supplied reagents.

---

## Before Starting

- The Resuspension Buffer (R3), Lysis Buffer (L7), and Precipitation Buffer (N3) provided in the kit are **not** used in this protocol. You will use Wash Buffer (W8), Elution Buffer (E4), and TE Buffer (TE) from the kit. You need to prepare the following solutions. Read the protocol carefully to determine the volume of each solution you need to prepare.

- M1: 3 M NaCl, 30% (w/v) PEG 8000
- M2: 100 mM Tris-HCl, pH 8.0, 25 mM EDTA
- M3: 4% SDS
- M4: 3 M potassium acetate, pH 5.5

Store solutions M1, M2, M3, and M4 at room temperature.

- Inoculate an aliquot of YT medium with 1/150 volume of lawn cells (a confluent culture of the bacterial host strain). Infect the cells with an M13 colony or a phage stock. Shake vigorously for **no longer** than 5 h (longer incubations may result in deletions).
  - Set a water bath or heat block to 70°C
- 

## Equilibrating the Column

Place the PureLink™ HiPure column on the PureLink™ Nucleic Acid Purification Rack (see the manual supplied with the rack for more details). Apply Equilibration Buffer (EQ1) to the column. Allow the solution in the column to drain by gravity flow.

	Miniprep	Midiprep	Maxiprep
Volume of EQ1	2 ml	10 ml	30 ml

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*Continued on next page*



# Procedure for ssM13 DNA, Continued

## Preparing Cell Lysate

1. Harvest the cells by centrifugation. *The ssM13 DNA is in the supernatant.* Transfer the supernatant to a new, sterile tube and centrifuge again to remove all traces of bacterial cells. Transfer the supernatant to a new, sterile tube.

	Miniprep	Midiprep	Maxiprep
Culture Volume	1-10 ml	10-25 ml	25-100 ml

2. To each 10 ml of supernatant, add 2 ml solution M1. Mix thoroughly and incubate on ice for 15 minutes to precipitate the M13 phage particles.
3. Collect the phage particles by centrifuging the sample at  $>10,000 \times g$  for 10 minutes. Discard the supernatant.
4. Resuspend the phage particles in solution M2 by pipetting up and down repeatedly.

	Miniprep	Midiprep	Maxiprep
Volume of M2	1 ml	3 ml	9 ml

5. Lyse the phage particles by adding solution M3. Mix thoroughly by inverting the tube several times. Incubate at  $70^{\circ}\text{C}$  for the time indicated in the table.

	Miniprep	Midiprep	Maxiprep
Volume of M3	1 ml	3 ml	9 ml
Incubation time	10 min	20 min	20 min

6. Add solution M4 to the lysate. Mix thoroughly by inverting the tube five times. Centrifuge at  $>12,000 \times g$  for 10 minutes at room temperature.

	Miniprep	Midiprep	Maxiprep
Volume of M4	1 ml	3 ml	9 ml

## Binding and Washing DNA

1. Load the supernatant from Step 6 (above) onto the equilibrated column. Allow the solution in the column to drain by gravity flow.
2. Wash the column **twice** with Wash Buffer (W8). Allow the solution in the column to drain by gravity flow after each wash. Discard the flow-through.

	Miniprep	Midiprep	Maxiprep
Volume of W8	2 x 2.5 ml	2 x 10 ml	2 x 60 ml

Continued on next page

## Procedure for ssM13 DNA, Continued

---

### Eluting and Precipitating DNA

1. Place a sterile centrifuge tube (elution tube) under the column.
2. Add Elution Buffer (E4) to the column to elute DNA. Allow the solution to drain by gravity flow. Do not force out any remaining solution. *The elution tube contains the purified DNA.* Discard the column.

	Miniprep	Midiprep	Maxiprep
Volume of E4	0.9 ml	5 ml	15 ml

3. Add 0.7 volumes of isopropanol to the elution tube. Mix well.

	Miniprep	Midiprep	Maxiprep
Volume of isopropanol	0.63 ml	3.5 ml	10.5 ml

4. Centrifuge the mixture at  $>12,000 \times g$  at  $4^{\circ}\text{C}$  for 30 minutes. Carefully remove and discard the supernatant.
5. Resuspend the DNA pellet in 70% ethanol.

	Miniprep	Midiprep	Maxiprep
Volume of 70% ethanol	1 ml	3 ml	5 ml

6. Centrifuge at  $>15,000 \times g$  at  $4^{\circ}\text{C}$  for 5-10 minutes. Carefully remove and discard the supernatant.
7. Air-dry the pellet for 10 minutes.
8. Resuspend the DNA pellet in TE Buffer (TE).

	Miniprep	Midiprep	Maxiprep
Volume of TE	10-60 $\mu\text{l}$	60-100 $\mu\text{l}$	100-400 $\mu\text{l}$

---

# Troubleshooting

## Introduction

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

Problem	Cause	Solution
Pipetting lysate	Pellet is viscous and does not adhere to tube	After centrifuging the lysate, allow the tube sit for 5 minutes to separate the clear lysate from the pellet (pellet may be floating). Remove the clear lysate to a fresh tube and centrifuge again to remove any remaining debris.
	Using a high volume of culture	Use the recommended culture volumes. If you are using higher culture volumes than the recommended volume, double the volumes of the Resuspension Buffer (R3), Lysis Buffer (L7) and Precipitation Buffer (N3) as designated in the protocol.
Low plasmid DNA yield	Buffers not stored correctly	Store Lysis Buffer (L7) and Equilibration Buffer (EQ1) at room temperature.
	Lysate centrifuged at 4°C	Make sure that the rotor and the centrifuge are at room temperature for the lysate centrifugation step.
	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 increase plasmid yield and quality.
	Lysate at improper pH or salt concentration to bind column	Make sure that the correct volume of Precipitation Buffer (N3) is added when neutralizing the lysate.
	Plasmid DNA pellet over-dried	Do not dry the DNA pellet with a vacuum system.

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## Troubleshooting, Continued

Problem	Cause	Solution
Slow column flow	Column clogged	Pipette the lysate supernatant onto the column. Do not pour the lysate onto the column, as some of the precipitate could enter the column.
Genomic DNA contamination	Genomic DNA sheared during handling	Gently invert tubes to mix after adding buffers. <b>Do not vortex</b> 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. Make sure that the lysate is not warmed above room temperature during the centrifugation.
	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.
	RNase A digestion incomplete	Make sure RNase A is added to Resuspension Buffer (R3). Use recommended volume of buffer R3. Make sure that buffer with RNase A is stored at 4°C.

# Technical Support

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## Web Resources



Visit the Invitrogen website at [www.invitrogen.com](http://www.invitrogen.com) for:

- Technical resources including manuals, vector maps and sequences, application notes, MSDSs, etc.
  - Complete technical support contact information.
  - Access to the Invitrogen Online Catalog.
  - Additional product information and special offers.
- 

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## MSDS

MSDSs (Material Safety Data Sheets) are available on our website at [www.invitrogen.com/msds](http://www.invitrogen.com/msds).

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## Certificate of Analysis

Certificate of Analysis (CofA) for the product is available on our website at [www.invitrogen.com/cofa](http://www.invitrogen.com/cofa).

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# Purchaser Notification

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