

# PureLink™ HiPure Plasmid DNA Purification Kits

For mini, midi, and maxi preparation of plasmid DNA

**Catalog Numbers** K2100-02, K2100-03, K2100-04, K2100-05, K2100 06, and K2100-07

**Revision** B.0

**Publication Number** MAN0000486

**For Research Use Only.** Not for use in diagnostic procedures.

Information in this document is subject to change without notice.

## **DISCLAIMER**

TO THE EXTENT ALLOWED BY LAW, LIFE TECHNOLOGIES AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

## **IMPORTANT LICENSING INFORMATION**

These products may be covered by one or more Limited Use Label Licenses. By use of these products, you accept the terms and conditions of all applicable Limited Use Label Licenses.

## **TRADEMARKS**

© 2015 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified. TaqMan is a registered trademark of Roche Molecular Systems, Inc., used under permission and license.

# Contents

Kit Contents and Storage .....	4
<b>Description of the System .....</b>	<b>7</b>
About the Kit .....	7
Experimental Overview .....	10
<b>Methods.....</b>	<b>11</b>
Before Starting .....	11
Miniprep Procedure .....	14
Midiprep Procedure .....	18
Maxiprep Procedure.....	23
Estimating DNA Yield and Quality .....	27
Expected Results .....	28
<b>Appendix.....</b>	<b>29</b>
Procedure for BAC DNA .....	29
Procedure for Bacmid DNA .....	33
Procedure for Cosmid DNA.....	35
Procedure for ssM13 DNA .....	38
Troubleshooting.....	41
Accessory Products.....	43
Technical Support .....	44

# 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 Mini and Midi Plasmid DNA Purification Kits are listed below.

Component Cat. No.	Miniprep		Midiprep	
	K2100-02	K2100-03	K2100-04	K2100-05
Resuspension Buffer (R3)	10 mL	50 mL	100 mL	200 mL
RNase A	100 µL	550 µL	550 µL	1.5 mL
Lysis Buffer (L7)	10 mL	50 mL	100 mL	200 mL
Precipitation Buffer (N3)	10 mL	40 mL	100 mL	200 mL
Equilibration Buffer (EQ1)	50 mL	250 mL	250 mL	2 × 250 mL
Wash Buffer (W8)	125 mL	500 mL	500 mL	2 × 500 mL
Elution Buffer (E4)	25 mL	90 mL	125 mL	250 mL
TE Buffer (TE)	15 mL	15 mL	15 mL	30 mL
HiPure Columns	25 each	100 each	25 each	50 each
Column Holders	—	—	5 each	10 each

## Kit Contents and Storage, Continued

---

### Contents, continued

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

Component Cat. No.	Maxiprep	
	K2100-06	K2100-07
Resuspension Buffer (R3)	100 mL	250 mL
RNase A	550 µL	1.5 mL
Lysis Buffer (L7)	100 mL	250 mL
Precipitation Buffer (N3)	100 mL	250 mL
Equilibration Buffer (EQ1)	300 mL	2 × 400 mL
Wash Buffer (W8)	2 × 300 mL	3 × 500 mL
Elution Buffer (E4)	200 mL	400 mL
TE Buffer (TE)	30 mL	30 mL
HiPure Columns	10 each	25 each
Column Holders	3 each	5 each

## Kit Contents and Storage, Continued

### 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 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

---

### System summary

The PureLink™ HiPure Plasmid Purification Kits allow you to isolate 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 28).

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 11).

---

### 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.

# About the Kit, Continued

---

## 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.

To purify plasmid DNA, you will harvest *E. coli* cells, resuspend the cells in Resuspension Buffer (R3) with RNase A, and then lyse the cells with Lysis Buffer (L7). Then, you will add Precipitation Buffer (N3) to the lysate and clarify the lysate using a centrifuge. Next, you will pass the clear lysate 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). After washing, you will elute the plasmid DNA under high salt conditions with the Elution Buffer (E4). Last, you will desalt and concentrate the DNA with an alcohol precipitation step. You can complete the entire protocol in 1.5–2 hours.

---

## Advantages

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

- Purify all types and sizes of plasmid DNA, including BAC, bacmids, and ssM13 DNAs (see page 29)
- Purify high-quality plasmid DNA suited for mammalian transfections
- High yield of plasmid DNA
- Reliable performance of the purified plasmid DNA in a variety of applications (see **Downstream applications**, page 9)

## About the Kit, Continued

### System specifications

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.

### Downstream applications

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

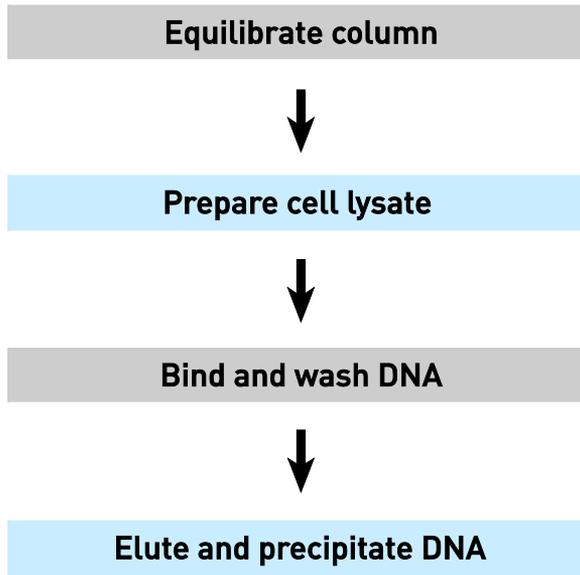
- Transfection of mammalian cells
- Automated and manual DNA sequencing
- PCR amplification
- *In vitro* transcription
- Bacterial cell transformation
- Cloning
- Labeling

# Experimental Overview

---

## Introduction

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



# Methods

## Before Starting

---

**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.

---

**Note** 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.

---

**Bacterial cultures** Grow transformed *E. coli* cells overnight in LB (Luria-Bertani) medium with the appropriate antibiotic. Harvest the bacterial culture in transition between exponential phase and stationary phase. The culture should have a cell density of  $\sim 10^9$  cells/mL or an optical density of 2.0 at 600 nm (OD<sub>600</sub>).

---

**Plasmid type and copy number** Use PureLink™ HiPure Plasmid DNA Purification Kits for purifying all types of plasmid DNA, including BAC (page 29), bacmids (page 33), and ssM13 DNAs (page 37). High copy number plasmids provide the best results, with a typical yield of 2–6 µg DNA/mL from an overnight culture. Yields from low copy number plasmids are dependent upon culture conditions and vector/host strain combinations. When isolating low copy number plasmids, use a higher volume of cell culture, as directed in the protocol. Recommended volumes of cell culture for plasmid DNA purification are listed in the table below.

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

\*When performing Maxipreps of low copy number plasmids from bacterial cultures of >200 mL, double the volumes of Resuspension Buffer (R3), Lysis Buffer (L7), and Precipitation Buffer (N3) indicated in the protocol (page 25).

## Before Starting, Continued

---

### Note

If the provided buffers in the Maxiprep kit are not sufficient due to the requirements of isolating low copy number plasmids, you may obtain additional buffers by ordering the PureLink™ HiPure BAC Buffer kit (see **Additional products**, page 43).

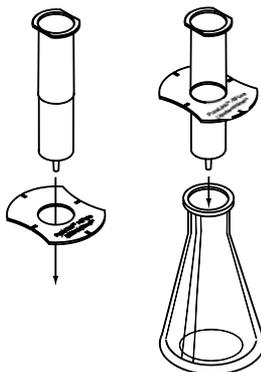
Follow the recommendations below to obtain the best results:

- Maintain a sterile workspace and equipment (including pipette tips and tubes) to avoid DNase contamination.
- Ensure that no DNase is introduced into the sterile solutions supplied with the kit.
- Use the PureLink™ Nucleic Acid Purification Rack for column purification (see **Purification rack**, page 13).
- Perform all recommended wash steps for best results.
- Use the TE Buffer (TE) provided or 10 mM Tris-HCl, pH 8.5 to resuspend the DNA pellet.

### Using the 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.

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.



## Before Starting, Continued

---

### Purification rack

The PureLink™ Nucleic Acid Purification Rack (see page 43) 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.

---

### Buffer preparation

#### 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.

---

### 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	14
Midiprep kit	18
Maxiprep kit	23
Purifying BAC DNA	29
Purifying Bacmid DNA	33
Purifying Cosmid DNA	35
Purifying ssM13 DNA	37

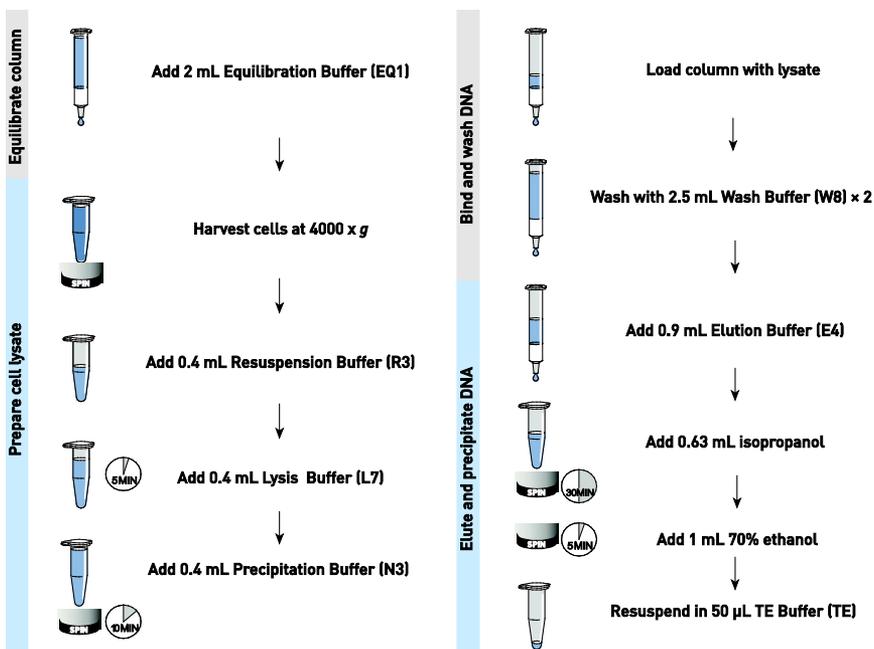
# Miniprep Procedure

## Introduction

The PureLink™ HiPure Plasmid DNA Miniprep Kit allows you to purify up to 30 µg of high quality plasmid DNA from 1–3 mL overnight *E. coli* cultures in ~1 hour when cloning high copy number plasmids.

## Experimental overview

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



# Miniprep Procedure, Continued

---

**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 13 for details.

**Materials needed**

- Overnight culture of transformed *E. coli* cells (page 11)
- Isopropanol
- 70% ethanol
- Sterile, microcentrifuge tubes
- PureLink™ Nucleic Acid Purification Rack (page 43)
- Microcentrifuge capable of centrifuging at >12,000 × g

**Components supplied with the kit**

- Resuspension Buffer (R3) with RNase A (page 13)
- Lysis Buffer (L7)
- Precipitation Buffer (N3)
- Equilibration Buffer (EQ1)
- Wash Buffer (W8)
- Elution Buffer (E4)
- TE Buffer (TE)
- PureLink™ HiPure Mini Columns

---

**Equilibrate the column** Place the PureLink™ HiPure Mini column on the PureLink™ Nucleic Acid Purification Rack (refer to 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 **Prepare cell lysate** (next page) while the column is equilibrating.

# Miniprep Procedure, Continued

---

## Prepare 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  $4000 \times g$  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 until the lysate mixture is thoroughly homogenous. **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 thoroughly homogeneous. **Do not vortex.**
6. Centrifuge the lysate at  $>12,000 \times g$  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 lysate and gelatinous pellet to separate. Pipet the clear lysate into another sterile tube and centrifuge at  $>12,000 \times g$  for 5 minutes at room temperature to remove any remaining cellular debris.
7. Proceed to **Bind and wash DNA** (next page).

## Miniprep Procedure, Continued

---

### Bind and wash DNA

1. Load the supernatant from **Prepare cell lysate**, step 6 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 **Elute and precipitate DNA**, below.
- 

### Elute and precipitate 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°C. Carefully remove and discard the supernatant.
  5. Resuspend the DNA pellet in 1 mL 70% ethanol.
  6. Centrifuge the tube at  $>12,000 \times g$  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 50  $\mu$ 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 for 1 minute at room temperature. Transfer the supernatant (DNA sample) into a fresh tube.
- 

### Store DNA

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.

---

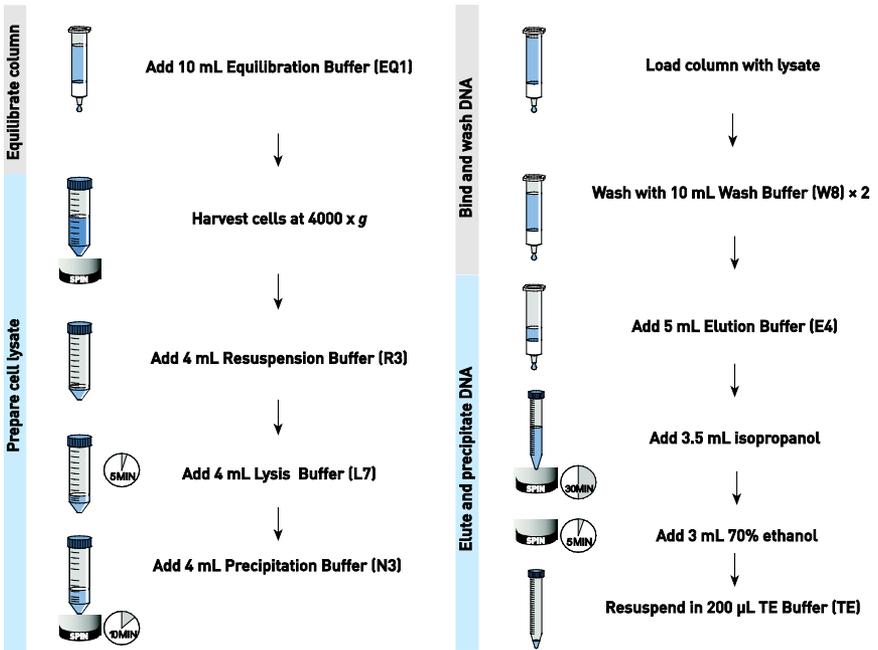
# Midiprep Procedure

## Introduction

The PureLink™ HiPure Plasmid Filter Midiprep Kit allows you to purify 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.

## Experimental overview

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



# Midiprep Procedure, Continued

---

**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 13 for details.

**Materials needed**

- Overnight culture of transformed *E. coli* cells (page 11)
- Isopropanol
- 70% ethanol
- Sterile, microcentrifuge tubes
- PureLink™ Nucleic Acid Purification Rack (page 43)
- Tubes or centrifuge bottles for harvesting cells
- Centrifuge and rotor appropriate for harvesting cells
- 15-mL centrifuge tubes (elution tubes) capable of withstanding centrifugation forces >12,000 × g
- Centrifuge capable of centrifuging at >12,000 × g at 4°C
- *Optional:* PureLink™ HiPure Precipitator Module (page 43)

**Components supplied with the kit**

- Resuspension Buffer (R3) with RNase A (page 13)
- Lysis Buffer (L7)
- Precipitation Buffer (N3)
- Equilibration Buffer (EQ1)
- Wash Buffer (W8)
- Elution Buffer (E4)
- TE Buffer (TE)
- PureLink™ HiPure Midi Columns
- Column Holder

---

**Equilibrate the column**

Use the Column Holder to support a HiPure Midi Column in a flask (see page 12), or place the 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 **Prepare cell lysate** (page 20), while the column is equilibrating.

# Midiprep Procedure, Continued

---

## Prepare cell lysate

1. For **high copy number plasmids**, use 15–25 mL of an overnight LB culture per sample in a disposable 50-mL conical 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 tube.
2. Harvest the cells by centrifuging the overnight LB culture at  $4000 \times g$  for 10 minutes. Remove all medium.
3. Add 4 mL Resuspension Buffer (R3) with RNase A to the cell pellet and resuspend the cells until homogeneous.
4. Add 4 mL Lysis Buffer (L7). Mix gently by inverting the capped tube until the lysate mixture is thoroughly homogenous. **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 thoroughly homogeneous. **Do not vortex.**
6. Centrifuge the mixture at  $>12,000 \times g$  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 lysate and gelatinous pellet to separate. Pipet the clear lysate into another, sterile tube and centrifuge at  $>12,000 \times g$  at room temperature for 5 minutes to remove any remaining cellular debris.
7. Proceed to **Bind and wash DNA**, next page.

# Midiprep Procedure, Continued

---

## **Bind and wash DNA**

1. Load the supernatant from **Prepare cell lysate**, step 6 (page 20) 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 **Elute and precipitate DNA**.
- 

## **Note**

For DNA precipitation, you can use the PureLink™ HiPure Precipitator Module (page 43) which allows you to precipitate DNA within 10 minutes without using a centrifuge. Alternatively, see **Elute and Precipitate DNA** on page 22 to perform traditional DNA precipitation using centrifugation.

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

# Midiprep Procedure, Continued

---

## Elute and precipitate DNA

1. Place a sterile 15-mL centrifuge tube (elution tube) under the column.
2. Add 5 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 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  $>12,000 \times g$  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  $>12,000 \times g$  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.

---

## Store DNA

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.

---

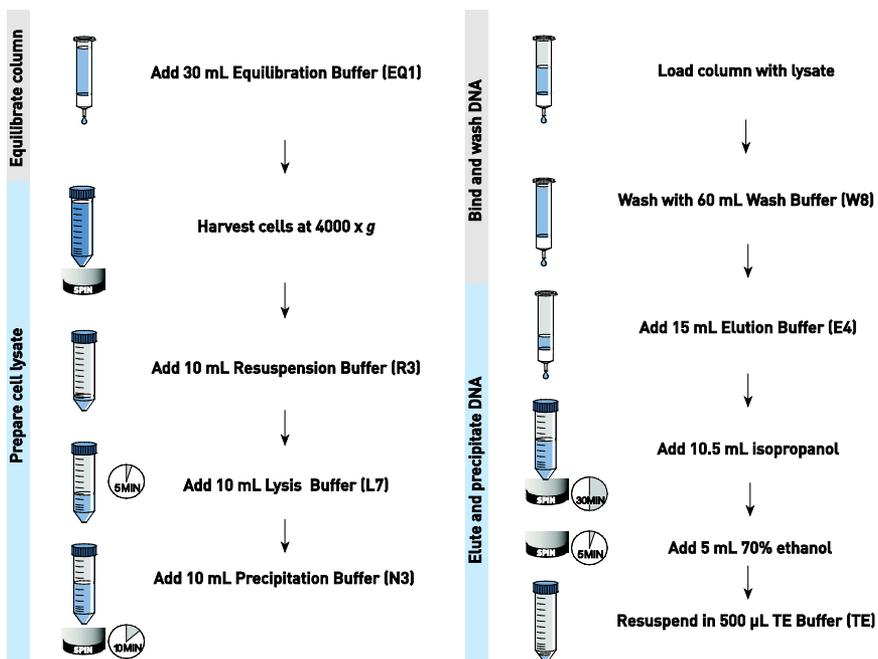
# Maxiprep Procedure

## Introduction

The PureLink™ HiPure Plasmid Filter Maxiprep Kit allows you to purify 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.

## Experimental overview

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



# Maxiprep Procedure, Continued

---

**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 13 for details.

**Materials needed**

- Overnight culture of transformed *E. coli* cells (page 11)
- Isopropanol
- 70% ethanol
- Sterile, microcentrifuge tubes
- PureLink™ Nucleic Acid Purification Rack (page 43)
- Tubes or centrifuge bottles for harvesting cells
- Centrifuge and rotor appropriate for harvesting cells
- Sterile 50-mL centrifuge tube (elution tube) capable of withstanding centrifugation forces >12,000 × g
- Centrifuge capable of centrifuging at >12,000 × g at 4°C
- *Optional:* PureLink™ HiPure Precipitator Module (page 43)

**Components supplied with the kit**

- Resuspension Buffer (R3) with RNase A (page 13)
- Lysis Buffer (L7)
- Precipitation Buffer (N3)
- Equilibration Buffer (EQ1)
- Wash Buffer (W8)
- Elution Buffer (E4)
- TE Buffer (TE)
- PureLink™ HiPure Maxi Columns
- Column Holder

---

**Equilibrate the column** Use the Column Holder to support a HiPure Maxi Column in a flask (see page 12), or place the Maxi Column on the PureLink™ Nucleic Acid Purification Rack (refer to 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 **Prepare cell lysate** (page 25) while the column is equilibrating.

# Maxiprep Procedure, Continued

---

## Prepare 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  $4000 \times g$  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 homogenous. **Do not vortex.** Incubate the tube 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. Centrifuge the mixture at  $>12,000 \times g$  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 lysate and gelatinous pellet to separate. Pipet the clear lysate into another tube and centrifuge the tube at  $>12,000 \times g$  for 5 minutes at room temperature to remove any remaining cellular debris.
7. Proceed to **Bind and wash DNA**.

---

## Bind and wash DNA

1. Load the supernatant from **Prepare cell lysate**, step 6 (page 25) 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 **Elute and precipitate DNA**.

# Maxiprep Procedure, Continued

---

## Note

To precipitate DNA, you can use the PureLink™ HiPure Precipitator Module (page 43) which allows you to precipitate DNA within 10 minutes without using a centrifuge. Alternatively, you can follow the protocol below to perform traditional DNA precipitation.

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

---

## Elute and precipitate 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  $>12,000 \times g$  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  $>12,000 \times g$  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.

---

## Store DNA

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

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 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.

### Qubit™ DNA Assay Kits

The Qubit™ DNA Assay Kits (page 43) 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 kits contain 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.

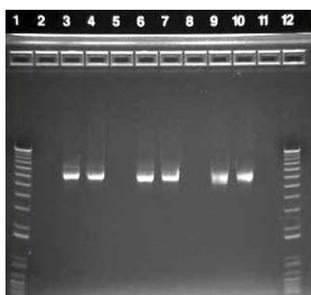
## Estimating DNA quality

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 substantially free of proteins that could interfere with downstream applications. The 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 Qubit™ Kit or by measuring UV absorbance at 260 nm

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

# Appendix

## Procedure for BAC DNA

---

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

**Note** Due to changes in the volumes of buffers used in this protocol, the 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 (see page 43 for ordering information).

**Before starting**

- Prepare a 20-hour culture of BAC containing bacteria in 2X YT medium and appropriate antibiotic. The absorbance at 600 nm of the final culture should be  $5.0 \pm 0.5$ .
- 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**.
- Warm Lysis Buffer (L7) briefly at 37°C to redissolve any particulate matter (see page 13).

## Procedure for BAC DNA, Continued

---

### Modified Column Wash Buffer

Isolating DNA from large amounts of bacterial culture increases the likelihood of residual RNA in the eluate. We recommend using a modified Column Wash Buffer (W8) with a higher salt concentration (850 mM NaCl) and lower pH (4.0) for more efficient removal of residual RNA (see **Bind and Wash DNA**, step 2 (page 31)).

#### Column Wash Buffer

100 mM sodium acetate, pH 4.0

850 mM NaCl

1. Prepare 100 mL of Modified Column Wash Buffer as follows:

Sodium acetate, anhydrous	0.82 g
Glacial acetic acid	1.43 mL
NaCl	4.97 g
Ultra pure water	80 mL
  2. Mix well and adjust with water to bring the final volume to 100 mL and check the pH. Slight variation for the final pH ( $\pm 0.1$ ) is possible and can be tolerated. Do not adjust the pH value using salt or acid.
  3. Store the buffer at room temperature.
- 

### Equilibrate the column

Support the column vertically using the PureLink™ Nucleic Acid Purification Rack (refer to the manual supplied with the rack for more details), or the Column Holder (for PureLink™ HiPure Midiprep and Maxiprep Columns, see page 12). Apply Equilibration Buffer (EQ1) to the column. Allow the solution in the column to drain by gravity flow.

Miniprep	Midiprep	Maxiprep
2 mL	10 mL	30 mL

## Procedure for BAC DNA, Continued

---

### Prepare cell lysate

1. Harvest the bacterial cells by centrifuging the overnight culture at  $9000 \times g$  for 15 minutes. Remove all medium.

Miniprep	Midiprep	Maxiprep
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.

Miniprep	Midiprep	Maxiprep
2 mL	8 mL	20 mL

3. Add Lysis Buffer (L7). Mix gently by inverting the capped tube until the mixture is thoroughly homogeneous. **Do not vortex.** Incubate at room temperature for 5 minutes.

Miniprep	Midiprep	Maxiprep
2 mL	8 mL	20 mL

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

Miniprep	Midiprep	Maxiprep
2 mL	8 mL	20 mL

5. Centrifuge the mixture at  $>12,000 \times g$  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 lysate and gelatinous pellet to separate. Pipet the clear lysate into a sterile tube and centrifuge the tube at  $>12,000 \times g$  for 5 minutes at room temperature to remove any remaining cellular debris.

---

### Bind and wash DNA

1. Load the supernatant from **Prepare cell lysate**, step 5 onto the equilibrated column. Allow the solution in the column to drain by gravity flow.
2. Wash the column with Wash Buffer (W8). Allow the solution in the column to drain by gravity flow. Discard the flow-through.

Miniprep	Midiprep	Maxiprep
2 × 2.5 mL	2 × 10 mL	1 × 60 mL

## Procedure for BAC DNA, Continued

---

### Elute and precipitate 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
0.9 mL	5 mL	15 mL

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

Miniprep	Midiprep	Maxiprep
0.63 mL	3.5 mL	10.5 mL

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

Miniprep	Midiprep	Maxiprep
1 mL	3 mL	5 mL

6. Centrifuge at  $>12,000 \times g$  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
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 you to purify a ~100 kb BAC molecule with yields of approximately 40  $\mu$ g DNA per 100 mL culture.

# Procedure for Bacmid DNA

---

## Introduction

The PureLink™ HiPure Plasmid DNA Miniprep Kit allows you to purify high quality Bacmid DNA (DH10Bac™) from *E. coli*. 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 hour and up to overnight.
  - Verify that the Resuspension Buffer (R3) contains RNase A, and warm Lysis Buffer (L7) briefly at 37°C to redissolve any particulate matter (see page 13).
- 

## Equilibrate the column

Place the PureLink™ HiPure Mini column on the PureLink™ Nucleic Acid Purification Rack (refer to 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.

---

## Prepare cell lysate

1. Harvest 1.5 mL bacterial cells by centrifuging the culture at 9000 × 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.
3. Add 0.4 mL Lysis Buffer (L7). Mix gently by inverting the capped tube until the mixture is thoroughly homogeneous. **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 thoroughly homogeneous. **Do not vortex.**
5. Centrifuge the mixture at >12,000 × 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 lysate and gelatinous pellet to separate. Pipet the clear lysate into a sterile tube and centrifuge at >12,000 × g for 5 minutes at room temperature to remove any remaining cellular debris.

## Procedure for Bacmid DNA, Continued

---

### Bind and wash DNA

1. Load the supernatant from **Prepare cell lysate**, step 5 (page 33) 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.
- 

### Elute and precipitate 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  $>12,000 \times g$  at 4°C for 20 minutes. Carefully remove and discard the supernatant.
5. Resuspend the DNA pellet in 1 mL 70% ethanol.
6. Centrifuge the tube at  $>12,000 \times g$  at 4°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  $\mu$ L TE Buffer (TE). Allow the pellet to dissolve for at least 10 minutes on ice. To avoid shearing the DNA, pipet only 1–2 times to resuspend.
9. Store the bacmid DNA at –20°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 hours (or overnight) on a 225 rpm shaking incubator.
- Verify that the Resuspension Buffer (R3) contains RNase A, and warm Lysis Buffer (L7) briefly at 37°C to redissolve any particulate matter (see page 13).

---

## Equilibrate the column

Support the column vertically using the PureLink™ Nucleic Acid Purification Rack (refer to the manual supplied with the rack for more details), or the Column Holder (for PureLink™ HiPure Midiprep and Maxiprep Columns, see page 12). Apply Equilibration Buffer (EQ1) to the column. Allow the solution in the column to drain by gravity flow.

Miniprep	Midiprep	Maxiprep
2 mL	10 mL	30 mL

## Procedure for Cosmid DNA, Continued

---

### Prepare cell lysate

1. Harvest bacterial cells by centrifuging the overnight culture at  $9000 \times g$  for 15 minutes. Remove **all** medium.

Miniprep	Midiprep	Maxiprep
1–3 mL	15–25 mL	100 mL

2. Add Resuspension Buffer (R3) containing **100  $\mu\text{g}/\text{mL}$**  RNase A to the pellet and resuspend the cells until homogeneous.

Miniprep	Midiprep	Maxiprep
0.4 mL	2 mL	8 mL

3. Add Lysis Buffer (L7). Mix gently by inverting the capped tube until the mixture is thoroughly homogeneous. **Do not vortex.** Incubate at room temperature for 5 minutes.

Miniprep	Midiprep	Maxiprep
0.4 mL	2 mL	8 mL

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

Miniprep	Midiprep	Maxiprep
0.4 mL	2 mL	8 mL

5. Centrifuge the mixture at  $>12,000 \times g$  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 lysate and gelatinous pellet to separate. Pipet the clear lysate into a sterile tube and centrifuge at  $>12,000 \times g$  at room temperature for 5 minutes to remove any remaining cellular debris.

### Bind and wash DNA

6. **Pipet** the supernatant from **Prepare cell lysate**, step 5 onto the equilibrated column. Allow the solution in the column to drain by gravity flow. 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.

Miniprep	Midiprep	Maxiprep
2 $\times$ 2.5 mL	2 $\times$ 10 mL	1 $\times$ 60 mL

## Procedure for Cosmid DNA, Continued

---

### Elute and precipitate 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.
7. *The elution tube contains the purified DNA.*  
Discard the column.

Miniprep	Midiprep	Maxiprep
0.9 mL	5 mL	15 mL

3. Add 0.7 volumes of isopropanol to the elution tube. Mix and place on ice for 10 minutes.

Miniprep	Midiprep	Maxiprep
0.63 mL	3.5 mL	10.5 mL

4. Centrifuge the mixture at  $>12,000 \times g$  at  $4^{\circ}\text{C}$  for 20 minutes. Carefully remove and discard the supernatant.
5. Resuspend the DNA pellet in 70% ethanol.

Miniprep	Midiprep	Maxiprep
1 mL	3 mL	5 mL

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). Allow the pellet to dissolve for at least 10 minutes on ice. To avoid shearing the DNA, pipet only 1–2 times to resuspend.

Miniprep	Midiprep	Maxiprep
10 $\mu\text{L}$	50–100 $\mu\text{L}$	200–400 $\mu\text{L}$

9. Store the cosmid DNA at  $-20^{\circ}\text{C}$  and avoid repeated freezing and thawing.
- 

### Expected results

This procedure allows you to purify 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 9 because of changes in the volumes used with the kit-supplied reagents.

- Before starting**
- The Resuspension Buffer (R3) and Lysis Buffer (L7) provided in the kit are **not** used in this protocol.
  - In addition to Precipitation Buffer (N3), Wash Buffer (W8), Elution Buffer (E4), and TE Buffer (TE) from the kit, the following solutions are required:
    - M1: 3 M NaCl, 30% (w/v) PEG 8000
    - M2: 100 mM Tris-HCl, pH 8.0, 25 mM EDTA
    - M3: 4% SDS
- Read the protocol carefully to determine the volume of each solution you need to prepare.
- Store solutions M1, M2, and M3, 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 hours (longer incubations may result in deletions).
  - Set a water bath or heat block to 70°C.

## Procedure for ssM13 DNA, Continued

### Equilibrate the column

Support the column vertically using the PureLink™ Nucleic Acid Purification Rack (refer to the manual supplied with the rack for more details), or the Column Holder (for PureLink™ HiPure Midiprep and Maxiprep Columns, see page 12). Apply Equilibration Buffer (EQ1) to the column. Allow the solution in the column to drain by gravity flow.

Miniprep	Midiprep	Maxiprep
2 mL	10 mL	30 mL

### Prepare cell lysate

8. Spin the culture using a centrifuge to sediment the bacterial cells. **The ssM13 DNA is in the supernatant.** Transfer the supernatant to a sterile tube and centrifuge to remove any traces of bacterial cells. Transfer the supernatant to a new, sterile tube.

Miniprep	Midiprep	Maxiprep
1–10 mL	10–25 mL	25–100 mL

1. 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.
2. Collect the phage particles by centrifuging the sample at  $>10,000 \times g$  for 10 minutes. Discard the supernatant.
3. Resuspend the phage particles in solution M2 by pipetting up and down repeatedly.

Miniprep	Midiprep	Maxiprep
1 mL	3 mL	9 mL

5. Lyse the phage particles by adding solution M3. Mix thoroughly by inverting the tube until the lysate is thoroughly homogeneous. Incubate the tube at 70°C for the time indicated in the table.

Miniprep	Midiprep	Maxiprep
1 mL	3 mL	9 mL
10 min	20 min	20 min

6. Add solution N3 to the lysate. Mix thoroughly by inverting the tube until the solution is thoroughly homogeneous. Centrifuge the tube at  $>12,000 \times g$  for 10 minutes at room temperature.

Miniprep	Midiprep	Maxiprep
1 mL	3 mL	9 mL

## Procedure for ssM13 DNA, Continued

---

### Bind and wash DNA

1. Load the supernatant from **Prepare cell lysate**, step 6 (page 39) onto the equilibrated column. Allow the solution in the column to drain by gravity flow.
2. Wash the column 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
2 × 2.5 mL	2 × 10 mL	1 × 60 mL

---

### Elute and precipitate DNA

1. Place a sterile centrifuge tube (elution tube) under the column.
9. 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
0.9 mL	5 mL	15 mL

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

Miniprep	Midiprep	Maxiprep
0.63 mL	3.5 mL	10.5 mL

3. Centrifuge the mixture at  $>12,000 \times g$  at 4°C for 30 minutes. Carefully remove and discard the supernatant.
4. Resuspend the DNA pellet in 70% ethanol.

Miniprep	Midiprep	Maxiprep
1 mL	3 mL	5 mL

5. Centrifuge at  $>12,000 \times g$  at 4°C for 5–10 minutes. Carefully remove and discard the supernatant.
6. Air-dry the pellet for 10 minutes.
7. Resuspend the DNA pellet in TE Buffer (TE).

Miniprep	Midiprep	Maxiprep
10–60 µL	60–100 µL	100–400 µL

---

# Troubleshooting

Observation	Cause	Solution
Pellet/debris is transferred onto column	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 (the 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.  If centrifugation at 4°C is unavoidable, transfer the cleared lysate to a new tube and warm it to room temperature in a water bath before loading it onto the column.
	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.

## Troubleshooting, Continued

Observation	Cause	Solution
Slow column flow	Column clogged	Pipet 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. 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.
	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.

## Accessory Products

---

### Additional products

The following products are also available from Thermo Fisher Scientific. For more details on these products, visit [thermofisher.com/lifescience](https://www.thermofisher.com/lifescience), or contact **Technical Support** (page 44).

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	K2100-14
	50 preps	K2100-15
PureLink™ HiPure Plasmid Filter Maxiprep Kits	10 preps	K2100-16
	25 preps	K2100-17
Expi293™ Expression System Kit	1 kit	A14635
PureLink™ HiPure Plasmid Filter and Precipitator Maxiprep Kit	10 preps	K2100-26
	25 preps	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	12795-027
	2.5 kg	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](https://www.thermofisher.com/lifescience), or contact **Technical Support** (page 44) 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](http://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](mailto: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 **[www.lifetechnologies.com/support](http://www.lifetechnologies.com/support)**

## Certificate of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available from **[www.lifetechnologies.com](http://www.lifetechnologies.com)**

## 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](http://www.lifetechnologies.com/termsandconditions)**. If you have any questions, please contact Life Technologies at **[www.lifetechnologies.com/support](http://www.lifetechnologies.com/support)**



**For support visit** [thermofisher.com/techresources](http://thermofisher.com/techresources) or  
email [techsupport@lifetech.com](mailto:techsupport@lifetech.com)

[thermofisher.com/lifescience](http://thermofisher.com/lifescience)

11 March 2015

**ThermoFisher**  
S C I E N T I F I C