LabNed Plasmid Purification Kits

A Novel Plasmid Purification System

Cat. nos. LN240004, LN240005, LN240007, LN240008

Rev. Date: 28 August 2017

User Manual

Table of Contents

Kit Contents and Storage

Types of Kits

This manual is supplied with the following products.

Product	Quantity	Cat. no.
LabNed Plasmid Midiprep Kit	50 preps	LN2400004
	250 preps	LN2400005
LabNed Plasmid Maxiprep Kit	20 preps	LN2400007
	100 preps	LN2400008

Intended Use

For research use only. Not intended for human or animal diagnostic or therapeutic uses.

Kit Contents and Storage, Continued

Midiprep and Maxiprep Kit Contents

The components included in the LabNed Plasmid Midiprep and Maxiprep Kits are listed below. **Store RNase A at 4°C and store all other components at room temperature.**

Item	**LN2400004**	**LN2400005**
	50 preps	250 preps
Cell Resuspending Buffer (E1)	220 mL	2 × 550 mL
RNase A (20 mg/mL)	1.5 mL	2 × 3 mL
Lysis Buffer (E2)	220 mL	1,100 mL
Precipitation Buffer (E3)	220 mL	1,100 mL
Equilibration Buffer (E4)	615 mL	3,200 mL
Wash Buffer (E5)	2 × 615 mL	2 × 3,200 mL
Elution Buffer (E6)	300 mL	1,650 mL
Columns	50 each	250 each

Item	**LN2400007**	**LN2400008**
	20 preps	100 preps
Cell Resuspending Buffer (E1)	220 mL	2 × 550 mL
RNase A (20 mg/mL)	1.5 mL	2 × 3 mL
Lysis Buffer (E2)	220 mL	1,100 mL
Precipitation Buffer (E3)	220 mL	1,100 mL
Equilibration Buffer (E4)	615 mL	3,200 mL
Wash Buffer (E5)	2 × 615 mL	2 × 3,200 mL
Elution Buffer (E6)	320 mL	1,650 mL
Columns	20 each	100 each

Kit Contents and Storage, Continued

Buffer Composition

The composition of buffers included in the LabNed Kits is listed below.

Buffer	Composition
Cell Resuspending Buffer (E1)	50 mM Tris-HCl, pH 8.0 10 mM EDTA
RNase A	20 mg/mL in 50 mM Tris-HCl, 10 mM EDTA, pH 8.0
Lysis Buffer (E2)	0.2 M NaOH 1% (w/v) SDS
Precipitation Buffer (E3)	3.1 M Potassium acetate, pH 5.5
Equilibration Buffer (E4)	0.1 M Sodium acetate, pH 5.0 0.6 M NaCl 0.15% (v/v) Triton® X-100
Wash Buffer (E5)	0.1 M Sodium acetate, pH 5.0 0.8 M NaCl
Elution Buffer (E6)	0.1 M Tris-HCl, pH 8.5 1.25 M NaCl

Introduction

System Summary

The LabNed Technology

LabNed Plasmid Purification Kits contain a unique anion exchange resin, supplied in disposable columns. The Midi and Maxi columns are used under gravity flow conditions without any further instrumentation. LabNed Plasmid Kits are the ideal tool to efficiently isolate ultrapure plasmid DNA within 45–60 minutes.

Procedure Overview

E. coli cells are harvested, resuspended in Cell Resuspending Buffer (E1) with RNase A, and then lysed with Lysis Buffer (E2). Precipitation Buffer (E3) 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 (E5). The plasmid DNA is eluted under high salt conditions with the Elution Buffer (E6).

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

System Summary, Continued

Advantages

Using the LabNed Plasmid Purification Kits to isolate plasmid DNA provides the following advantages:

- Unique anion exchange resin technology to isolate plasmid DNA negates the need for any organic solvents, cesium chloride (CsCl), or vacuum manifolds
- High yield of plasmid DNA (see next page)
- Purified DNA demonstrates improved performance in downstream applications (see next page)
- Purification of all types and sizes of plasmid DNA, including BAC and cosmid DNA (see pages 23–29), as well as ssM13 DNAs, λ-DNA, and plasmid DNA from fungi
- Minimal contamination with RNA

System Summary, Continued

System Specifications

Specification*	Midiprep	Maxiprep
Starting culture volume	15–25 mL	100 mL
Column Binding Capacity**	350 µg	850 µg
Column Reservoir Capacity	10 mL	60 mL
Elution Volume	5 mL	15 mL
DNA Recovery	85–90%	85–90%
Expected DNA Yield***	45–100 µg	300–500 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, plasmid type and size, bacterial strain, and growth conditions.

Downstream Applications

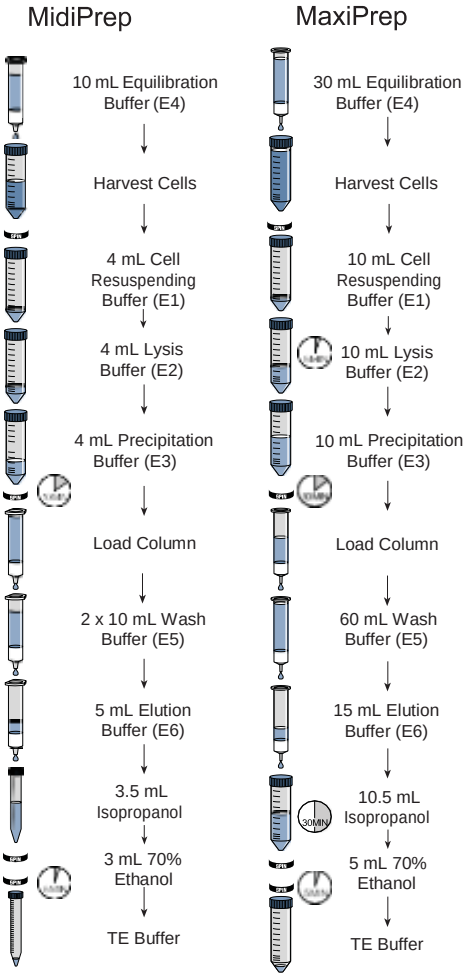
DNA purified using LabNed Plasmid Purification Kits is at a purity equivalent to two passes through CsCl gradients and is suitable for downstream applications, including those requiring the highest purity, such as:

- Transfection of mammalian cells
- Microinjection
- Vaccination
- Automated and manual DNA sequencing
- PCR amplification
- *In vitro* transcription
- Cloning

Experimental Overview

Introduction

The flow chart for purifying plasmid DNA using the LabNed Plasmid 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.

Some buffers in the LabNed Plasmid Purification Kits contain hazardous chemicals.

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

Plasmid Yields

All types and sizes of plasmid DNA can be prepared, but yields depend on the type of plasmid, copy number (low/medium/high), the bacterial strain, and the volume of bacterial culture used.

LabNed Plasmid Purification Kits are preferentially designed to extract and purify high copy plasmid DNA from *E. coli* cultures. Low copy plasmids can be prepared as well, but larger culture volumes will be necessary.

Although the LabNed system is compatible with other growth media, including rich media, we strongly recommend growing transformed *E. coli* cells overnight in buffered LB (Luria-Bertani) media for optimal results. The cell density should be approximately 1×10^9 cells per mL medium (1–1.5 A_{600} units/mL).

Before Starting, Continued

Plasmid Type and Copy Number

Use a high copy number plasmid to obtain a good yield of plasmid DNA. If you are using a low copy number plasmid, use a higher volume of cell culture, as directed in the protocol. High copy number plasmids typically yield >3–5 µg DNA/mL LB culture grown overnight; whereas low copy number plasmids typically yield 0.2–1 µg DNA/mL LB culture grown overnight.

The table below lists the starting culture volumes recommended for Midiprep, and Maxiprep plasmid DNA purification based on the plasmid copy number.

Plasmid Copy Number	Midiprep	Maxiprep
High copy number lasmid	15–25 mL	100 mL
Low copy number plasmid	25–100 mL	250–500 mL

Specific Protocols

The LabNed Plasmid Purification Kits may be used to purify all types and sizes of plasmid DNA, including BAC and cosmid DNA. Specific protocols for plasmid DNA purification using the various kits are described in this manual (see table below for page references).

Protocol	Page no.
Purifying plasmid DNA using:	
Midiprep kit	8
Maxiprep kit	13
Procedure for BAC and cosmid DNA	19

Before Starting, Continued

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 buffers supplied with the kit.
- Sterilize all equipment coming in contact with DNA, including pipette tips and tubes.
- Perform the recommended wash steps during purification to obtain the best results.
- Use TE Buffer or 10 mM Tris-HCl, pH 8.5 to resuspend the DNA pellet.

Preparing Buffers

Cell Resuspending Buffer (E1)

Add RNase A to the Cell Resuspending Buffer (E1) according to the note with the RNase A tube. Mix well. The final RNase A concentration is 100 µg/mL. Mark the bottle label to indicate that RNase A is added. Store the buffer with RNase A at 4°C.

Lysis Buffer (E2)

Check the Lysis Buffer (E2) for precipitates. If present, warm the solution briefly at 37°C to dissolve the precipitate.

Midiprep Procedure

Introduction

Up to 100 µg of high-quality plasmid DNA may be purified with the LabNed Plasmid Midiprep Kit in ~2 hours from 15–25 mL overnight *E. coli* cultures, when using high copy number plasmids.

Before Starting

Before beginning, verify that RNase A is added to the Cell Resuspending Buffer (E1) and that no precipitate has formed in the Lysis Buffer (E2), page 7.

Materials Supplied by the User

- Overnight culture of transformed *E. coli* cells
- Tubes or buckets of appropriate size for harvesting cells
- Centrifuge and rotor appropriate for harvesting cells
- Isopropanol
- 70% ethanol
- Sterile microcentrifuge tubes
- Appropriate 15 mL centrifuge tubes capable of withstanding centrifugation forces > 12,000 × *g*
- Centrifuge capable of centrifuging at >12,000 × *g* at 4°C

Components Supplied with the Kit

- Cell Resuspending Buffer (E1) with RNase A (page 7)
- Lysis Buffer (E2)
- Precipitation Buffer (E3)
- Equilibration Buffer (E4)
- Wash Buffer (E5)
- Elution Buffer (E6)
- LabNed Midi Columns

Midiprep Procedure, Continued

Equilibrating the Column

Place the LabNed Midi Column on the Rack Apply 10 mL Equilibration Buffer (E4) to the column. Allow the solution in the column to drain by gravity flow. Equilibration takes 10–15 minutes, but does not influence the speed of the protocol; therefore, proceed to Step 1 (below) during equilibration.

Overnight Culture

- For **high copy number plasmids**, use 15–25 mL of an overnight LB culture per sample in a 50-mL disposable tube.
- For **low copy number plasmids**, use 25–100 mL of an overnight LB culture per sample.

  Note: If you are using >25 mL of culture volume of high copy plasmids, add twice the amount of Cell Resuspending Buffer (E1) with RNase A, Lysis Buffer (E2), and Precipitation Buffer (E3) as directed in Steps 2, 3, and 4, below and next page, for best results.

Preparing Cell Lysate

1. Harvest the cells by centrifuging the overnight LB-culture at 4,000 × *g* for 10 minutes in a bucket. Remove all medium.
2. Add 4 mL Cell Resuspending Buffer (E1) with RNase A to the cell pellet and resuspend the cells until homogeneous. Transfer the cell suspension to a 15 mL centrifuge tube.

   Note: If overnight culture volume is >25 mL, use 8 mL Buffer E1.

Midiprep Procedure, Continued

Preparing Cell Lysate, Continued

3. Add 4 mL Lysis Buffer (E2). Mix gently by inverting the capped tube until a homogenous mixture is obtained. **Do not vortex.** Incubate at room temperature for 5 minutes.

   Note: Do not allow lysis to proceed for more than 5 minutes. If overnight culture volume is >25 mL, use 8 mL Buffer E2.

4. Add 4 mL Precipitation Buffer (E3) and mix immediately by inverting the capped tube until the mixture is homogeneous. The viscous matter present after cell lysis (Step 3) should not remain. **Do not vortex**.

   Note: When many samples are prepared in parallel, mix each sample immediately after adding Buffer E3. If overnight culture volume is >25 mL, use 8 mL Buffer E3.

5. Centrifuge the mixture 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 pellet. Pipette the clear lysate into another sterile tube and centrifuge at >12,000 × *g* for 5 minutes at room temperature to remove any remaining cellular debris.

6. Proceed to **Binding and Washing DNA**, below.

Binding and Washing DNA

1. Load the supernatant from Step 5 (above) onto the equilibrated column with a pipette. Allow the solution in the column to drain by gravity flow.
2. Wash the column **twice** with 10 mL Wash Buffer (E5). 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.

Eluting and Precipitating DNA

1. Place a sterile microcentrifuge tube (elution tube) under the column.
2. Add 5 mL Elution Buffer (E6) 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.
4. Centrifuge the elution tube at >12,000 × g for 30 minutes at 4°C. Carefully remove and discard the supernatant.
5. Wash the DNA pellet in 3 mL 70% ethanol.
6. Centrifuge at >12,000 × g for 5 minutes at 4°C. Carefully remove and discard the supernatant.
7. Air-dry the pellet for 10 minutes.

   Note: Be careful when drying the DNA pellet under vacuum in a speed-vac or in a vacuum chamber using an oil pump or a water jet filter pump. If the pellet is overdried, the DNA will not redissolve completely.

Midiprep Procedure, Continued

Eluting and Precipitating DNA, Continued

8. Resuspend the DNA pellet in a suitable volume of buffer (i.e., 10 mM Tris-HCl, pH 8.0, TE Buffer, or water).

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

Storing DNA

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

Up to 500 µg of high-quality plasmid DNA may be purified with the LabNed Plasmid Maxiprep Kit in ~2 hours from 100 mL overnight *E. coli* cultures, when using high copy number plasmids.

Before Starting

Before beginning, verify that RNase A is added to the Cell Resuspending Buffer (E1) and that no precipitate has formed in the Lysis Buffer (E2), see page 7.

Materials Supplied by the User

- Overnight culture of transformed *E. coli* cells
- Isopropanol
- 70% ethanol
- Sterile microcentrifuge tubes
- Appropriate 50 mL centrifuge tube capable of withstanding centrifugation forces > 12,000 × *g*
- Centrifuge capable of centrifuging at >12,000 × *g* at 4°C

Components Supplied with the Kit

- Cell Resuspending Buffer (E1) with RNase A (page 7)
- Lysis Buffer (E2)
- Precipitation Buffer (E3)
- Equilibration Buffer (E4)
- Wash Buffer (E5)
- Elution Buffer (E6)
- LabNed Maxi Columns

Maxiprep Procedure, Continued

Equilibrating the Column

Place the LabNed Maxi Column on the Rack. Apply 30 mL Equilibration Buffer (E4) to the column. Allow the solution in the column to drain by gravity flow. Equilibration takes about 10–15 minutes, but does not influence the speed of the protocol; therefore, proceed to Step 1 (below) during equilibration.

Overnight Culture

- For **high copy number plasmids**, use 100 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 Cell Resuspending Buffer (E1) with RNase A, Lysis Buffer (E2), and Precipitation Buffer (E3) as directed in Steps 2, 3, and 4, below and next page.

Preparing Cell Lysate

1. Harvest the cells by centrifuging the overnight LB-culture at 4,000 × *g* for 10 minutes in a bucket. Remove all medium.
2. Add 10 mL Cell Resuspending Buffer (E1) with RNase A to the pellet and resuspend the cells until homogeneous. Transfer cell suspension to a 50-mL centrifuge tube.

   Note: If overnight culture volume is >200 mL, use 20 mL Buffer E1.
3. Add 10 mL Lysis Buffer (E2). Mix gently by inverting the capped tube until a homogenous mixture is obtained. **Do not vortex.** Incubate at room temperature for 5 minutes.

   Note: Do not allow lysis to proceed for more than 5 minutes. If overnight culture volume is >200 mL, use 20 mL Buffer E2.

Preparing Cell Lysate, Continued

4. Add 10 mL Precipitation Buffer (E3) and mix immediately by inverting the capped tube until the mixture is homogeneous. The viscous matter present after cell lysis (Step 3) should not remain. Do not vortex.

   Note: When many samples are prepared in parallel, each sample should be mixed immediately after adding Buffer E3. If overnight culture volume is >200 mL, use 20 mL Buffer E3.

5. Centrifuge the mixture 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 pellet. Pipette the clear lysate into another sterile tube and centrifuge at >12,000 × *g* for 5 minutes at room temperature to remove any remaining cellular debris.

6. Proceed to **Binding and Washing DNA**, below.

Binding and Washing DNA

1. Load the supernatant from Step 5 (above) onto the equilibrated column with a pipette. Allow the solution in the column to drain by gravity flow.
2. Wash the column with 60 mL Wash Buffer (E5). Allow the solution in the column to drain by gravity flow. Discard the flow-through.
3. Proceed to **Eluting and Precipitating DNA** (next page).

Eluting and Precipitating DNA

1. Place a sterile 50-mL centrifuge tube (elution tube) under the column.
2. Add 15 mL Elution Buffer (E6) to the column. Allow the solution to drain by gravity flow. Do not force out any remaining solution. Discard the column.

   The elution tube contains the purified DNA.
3. Add 10.5 mL isopropanol to the elution tube. Mix well.
4. Centrifuge at >12,000 × *g* for 30 minutes at 4°C. Carefully remove and discard the supernatant.
5. Wash the DNA pellet in 5 mL 70% ethanol.
6. Centrifuge the elution tube at >12,000 × *g* for 5 minutes at 4°C. Carefully remove and discard the supernatant.
7. Air-dry the pellet for 10 minutes.

   Note: Be careful when drying the DNA pellet under vacuum in a speed-vac or in a vacuum chamber using an oil pump or a water jet filter pump. If the pellet is overdried, the DNA will not redissolve completely.
8. Resuspend the DNA pellet in a suitable volume of buffer (i.e. 10 mM Tris-HCl, pH 8.0, TE Buffer or water).

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

Storing DNA

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

DNA Yield

Perform DNA quantitation using UV absorbance at 260nm.

UV Absorbance

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

   DNA (µg/mL) = $A_{260} \times 50 \times$ dilution factor

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

Expected Results

Summary of Expected Results

The summary of results using the LabNed 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:	**Midiprep**	**Maxiprep**
Processing Time	~2 hours	~2 hours
Plasmid DNA Yield	100 µg	500 µg
Endotoxin	<0.1 EU/µg	<0.1 EU/µg
Automated Sequencing	Successful	Successful
Restriction Enzyme Digestion	Successful	Successful

Appendix

Procedure for BAC and cosmid DNA

BAC and cosmid DNA

LabNed Plasmid Purification Kits allow you to purify high quality BAC (bacterial artificial chromosome) and cosmid DNA as well as PAC, YAC, or P1 constructs from *E. coli* cultures (see pages 23–29). To purify λ-DNA, ssM13 DNA, and plasmid DNA from fungi using LabNed Plasmid Purification Kits, download protocols at www.labned.com or contact Technical Support.

Since BAC, cosmid, PAC, YAC, and P1 DNA species behave like *very low copy* plasmids (in most cases present with 1 copy per cell), it is necessary to work with large volumes of culture (up to 500 mL) to obtain a reasonable DNA yield. Therefore, we recommend using the LabNed Plasmid Maxiprep Kits. To scale down the procedure to the Mini- or Midi-scale, use the amounts of bacterial culture mentioned in the protocol below.

Materials Supplied by the User

- Overnight culture of transformed *E. coli* cells
- Modified Wash Buffer (E5-850, pH 4.0), see page 26
- Isopropanol
- 70% ethanol
- Sterile microcentrifuge tubes
- Tubes or buckets of appropriate size for harvesting cells
- Centrifuge and rotor appropriate for harvesting cells
- Appropriate 50 mL centrifuge tube capable of withstanding centrifugation forces > 12,000 × *g*
- Centrifuge capable of centrifuging at >12,000 × *g* at 4°C

Procedure for BAC and cosmid DNA,
Continued

Components Supplied with the Kit

- Cell Resuspending Buffer (E1) with RNase A (page 25)
- Lysis Buffer (E2)
- Precipitation Buffer (E3)
- Equilibration Buffer (E4)
- Elution Buffer (E6)
- LabNed Maxi Columns

Media Requirements

In addition to LB broth, LabNed Plasmid Purification Kits are compatible with other media that are commonly used for the propagation of *E. coli* (i.e., Terrific Broth, or 2X YT).

Due to increased volumes used in this protocol, the amount of buffers provided in the LabNed Plasmid Purification Kits may not be sufficient to utilize all of the columns provided. For your convenience, buffers may be purchased separately.

Because large DNA species are "sticky", they will spread over the wall of the centrifuge tube if a fixed angle rotor is used. Therefore, a swing-out-rotor (i.e. HB-4 or HB-6 for Sorvall centrifuges) is recommended. If a swing-out-rotor is not available, siliconize the centrifuge tubes (i.e., Corex) with dimethyldichlorosilane.

Procedure for BAC and cosmid DNA,
Continued

Contaminating RNA

Using large amounts of bacterial culture increases the likelihood of residual RNA in the eluate. Follow the hints and suggestions below for mitigating RNA contamination.

- Normally, 100 µg/mL RNase A is sufficient to eliminate RNA (see Step 2, Preparing Cell Lysate, page 27). However, for the highest amounts of bacterial culture suggested (i.e., 500 mL for a Maxiprep), increase the amount of RNase A in Buffer E1 to 400 µg/mL and add 100 U/mL of RNase T1. The combined activities of RNase A and T1 result in better digestion efficiency.
- The higher salt concentration (850 mM NaCl instead of 800 mM) and the lower pH value (4.0) in the modified Wash Buffer (E5-850, pH 4.0), lead to a more efficient removal of residual RNA without affecting the DNA yield significantly (see page 26 for recipe).

Elution Buffer

Because of the large size of the constructs, it is useful to prewarm Buffer E6 to 50°C for elution.

Before Starting

- Reconstitute Cell Resuspending Buffer (E1) with the RNase A stock solution (20 mg/mL, provided with the kit). See **Contaminating RNA**, above.
- Inoculate a bacterial culture with the appropriate antibiotic and grow the bacteria for 14–16 h.
- Use the following amounts of bacterial culture:

Prep Size	Culture volume
Midi	Up to 100 mL
Maxi	300-500 mL

Procedure for BAC and cosmid DNA,
Continued

Recipe for Modified Wash Buffer: (E5-850, pH 4.0)

Modified Wash Buffer (E5-850, pH 4.0)

100 mM sodium acetate, pH 4.0
850 mM NaCl

1. Mix the following components to prepare 1 L Buffer E5-850, pH 4.0:

Sodium acetate	8.2 g
Glacial acetic acid	15 g
NaCl	49.67 g
Ultra pure water	800 mL

2. Mix well and adjust with water to bring the final volume to 1 liter. Check the pH. The final pH may differ slightly (± 0.1) depending on the pH meter used and such slight deviations can be tolerated. Do not adjust the pH value using salt or acid.
3. Store the buffer at room temperature.

Equilibrating the Column

Apply Equilibration Buffer (E4) to the column. Allow the solution in the column to drain by gravity flow.

Reagent	Midiprep	Maxiprep
Equilibration Buffer (E4)	10 mL	30 mL

Procedure for BAC and cosmid DNA,
Continued

Preparing Cell Lysate

1. Harvest the cells by centrifuging the overnight culture at 4,000 × *g* for 10 minutes in a bucket. Remove all medium.
2. Add Cell Resuspending Buffer (E1) containing RNase A to the pellet and resuspend the cells until homogeneous (no cell clumps should be visible). Transfer cell suspension to a centrifuge tube.

Reagent	Midiprep	Maxiprep
Cell Resuspending Buffer (E1)	8 mL	20 mL

3. Add Lysis Buffer (E2). Mix gently by inverting the capped tube until a homogenous mixture is obtained. Do not vortex. Incubate at room temperature for 5 minutes.

Reagent	Midiprep	Maxiprep
Lysis Buffer (E2)	8 mL	20 mL

4. Neutralize with Precipitation Buffer (E3). Mix gently but thoroughly until a homogeneous mixture is obtained. Do not vortex.

Reagent	Midiprep	Maxiprep
Precipitation Buffer (E3)	8 mL	20 mL

5. Centrifuge the mixture at $\geq$12,000 × *g* at room temperature for 10 minutes. Transfer the supernatant into a fresh tube.
6. Proceed to **Binding and Washing DNA,** next page.

Procedure for BAC and cosmid DNA,
Continued

Binding and Washing DNA

1. Load the supernatant from Step 5 (page 27) onto the equilibrated column. Allow the solution in the column to drain by gravity flow. Do not force out remaining buffer.
2. Wash the LabNed Column with the following volumes of Modified Wash Buffer (E5-850, pH 4.0):

Reagent	Midiprep	Maxiprep
Modified Wash Buffer (E5-850, pH 4.0)	2 × 10 mL	2 × 30 mL

3. Proceed to **Eluting and Precipitating DNA**, below.

Eluting and Precipitating DNA

1. Place a sterile centrifuge tube under the column.
2. Add Elution Buffer (E6) 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.

Reagent	Midiprep	Maxiprep
Elution Buffer (E6)	5 mL	15 mL

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

Reagent	Midiprep	Maxiprep
Isopropanol	3.5 mL	10.5 mL

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

Procedure for BAC and cosmid DNA,
Continued

Eluting and Precipitating DNA, Continued

5. Wash the DNA pellet in 70% ethanol.

Reagent	Midiprep	Maxiprep
Volume of 70% ethanol	3 mL	5 mL

6. Centrifuge at >12,000 × *g* for 5 minutes at 4°C. Carefully remove and discard the supernatant.
7. Air-dry the pellet for 5–10 minutes.
8. Resuspend the DNA pellet in 10 mM Tris-HCl (pH 8.5), TE Buffer, or water.

Reagent	Midiprep	Maxiprep
TE Buffer	200 µL	500 µL

Storing 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.

Troubleshooting

Introduction

Review the information below to troubleshoot your experiments with LabNed Plasmid Purification Kits.

A Diagnosis Protocol is useful for determining the cause of *low* or *negative* yields. By precipitating DNA after key steps of the purification protocol you may track DNA loss. The Diagnosis Protocol can be downloaded at www.labned.com.

Problem	Cause	Solution
Pipetting lysate	Pellet is viscous and does not adhere to tube	After centrifuging the lysate, allow the tube to sit for 5 minutes. Transfer 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.
Low plasmid DNA yield	Temperature of Buffers E1–E6 too low	Store Buffers E1–E6 at room temperature.
	Lysate centrifuged at 4°C	Use the rotor and centrifuge at room temperature. Alternatively, if the lysate is centrifuged at 4°C, warm the cleared lysate to room temperature (18°C to 25°C) before loading onto the LabNed Column.

Troubleshooting, Continued

Problem	Cause	Solution
Low plasmid DNA yield	**Low copy number plasmid.** Plasmid quantity in *E. coli* cells is dependent on the individual host-plasmid system	Increase the volume of starting culture. Carefully remove all medium before resuspending cells. Doubling the volumes of the Cell Resuspending Buffer (E1), Lysis Buffer (E2), and Precipitation Buffer (E3) may increase plasmid yield and quality.
	Plasmid DNA pellet over-dried	Air dry the DNA pellet. Drying the DNA pellet under vacuum in a speed-vac or in a vacuum chamber using an oil pump or a water jet filter pump may cause overdrying of the DNA pellet.
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. **Do not vortex** during Steps 3 and 4 of **Preparing Cell Lysate** as it can shear genomic DNA.
Additional plasmid forms present	**Plasmid DNA permanently denatured,** evident as a band migrating faster than supercoiled DNA on an agarose gel	Incubate the lysate, after the addition of Buffer E2 in Step 3 of **Preparing Cell Lysate,** at room temperature for no longer than 5 minutes.

Problem	Cause	Solution
Insoluble particles	If a few particles are visible with the redissolved plasmid DNA, the quality of the DNA is not compromised	Centrifuge the plasmid DNA solution for 1 minute and transfer the supernatant into a new tube.
RNA contamination	Lysate at improper pH or salt concentration	Avoid changes in conditions and volumes of Buffers E1–E6.
	Lysate left on column too long	Once the lysate is loaded onto the column, avoid delays in processing.
	Lysate droplets remaining on walls of column at elution	Wash away droplets of lysate from the walls of the column with Wash Buffer (E5). Allow Buffer E5 to run completely through the column before the second round of E5 or the Elution Buffer (E6) is applied.
	Lysate at improper temperature	Make sure that the lysate is not warmed above room temperature during centrifugation.
	RNase A digestion incomplete	Make sure that RNase A is added to Cell Resuspending Buffer (E1) and that it has been stored at 4°C for no longer than 6 months. Use recommended volume of Buffer E1.
	Host strain extremely rich of RNA	Use Modified Wash Buffer (E5-850, pH 4.0) instead of standard Wash Buffer (page 26).

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