invitrogen™

PureLink™ Pro Quick96 Plasmid Purification Kit

For high-throughput purification of plasmid DNA

Catalog nos. K2110-04A, K2110-24A

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Part no. 100007357

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User Manual

Contents

Experienced Users Procedure

Introduction

This quick reference sheet is provided for experienced users of the PureLink™ Pro Quick96 Plasmid Purification Kits. First time users should refer to the detailed protocols in this manual.

Step	Action
Grow Cells	Grow cells in ≤3 mL of LB medium with the appropriate antibiotic at 37ºC with shaking (200–250 rpm) in a PureLink™ Pro 96 Growth Block to 10^9 cells/mL (OD_{600} =1–2).
Harvest Cells and Prepare Lysates	1. Harvest cells by centrifugation at 2,100 × g for 10 minutes. Remove the growth media. 2. Resuspend cell pellets in 250 µl Resuspension Buffer with RNase A (see **Before Starting**). 3. Add 250 µl Lysis Buffer to each well. Mix by gently shaking, **do not** vortex or pipet to mix. 4. Incubate at room temperature for 2–5 minutes. **Do not exceed 5 minutes.** 5. Add 350 µl Neutralization Buffer to each well. Mix by **gently** pipetting up and down. 6. Proceed to **Purification Procedure with Centrifugation** (page vi), or **Purification Procedure with Vacuum** (page vii).

Continued on next page

Experienced Users Procedure, Continued

Step	Action
Purification Procedure with Centrifugation	Perform all centrifugation steps at 25°C. 1. Transfer 700–850 µl of each lysate to the PureLink™ 96 Pro Clarification Plate. Cover unused wells with Foil Tape.. 2. Place the Clarification Plate onto a 96-well, Deep-Well Block and centrifuge at ≥2,100 × g for 2 minutes. 3. Place a PureLink™ 96 Well Plasmid Filter Plate on a new Deep-Well Block. 4. Transfer the filtered lysate from the Deep-Well Block (Step 2) into the Plasmid Filter Plate and centrifuge at ≥2,100 × g for 2 minutes. Discard the flow-through. 5. *Optional* (*for EndA⁺ strains*): Add 600 µl Wash Buffer I with isopropanol into each well of the Plasmid Filter Plate and centrifuge at ≥2,100 × g for 2 minutes. Discard the flow-through. 6. ***Required***: Add 900 µl Wash Buffer II with ethanol into the wells of the Plasmid Filter Plate and centrifuge at ≥2,100 × g for 2 minutes. Discard the flow-through. 7. Repeat Step 6 above once. 8. Centrifuge the Plasmid Filter Plate at ≥2,100 × g for 10 minutes, and blot any residual liquid from the bottom of the plate. 9. Place the Plasmid Filter Plate onto a new PureLink™ 96 Pro Elution Plate. 10. Add 50–175 µl Elution Buffer and incubate the plate for 1 minute at room temperature. 11. Centrifuge at ≥2,100 × g for 1–2 minutes. 12. Seal the elution plate with Foil Tape (included with kit) and store plasmid DNA at –20ºC (long-term), or 4ºC (short-term), or proceed to the downstream application.

Continued on next page

Experienced Users Procedure, Continued

Step	Action
Purification Procedure with Vacuum	1. Assemble the vacuum manifold. Use a vacuum pressure of –12 to –15 in. Hg for the purification steps, or reduce the vacuum pressure until a flow rate of 1–2 drops per second is attained. 2. Place the PureLink™ 96 Well Plasmid Filter Plate inside the vacuum manifold base. Place the manifold lid on top and insert a PureLink™ Pro 96 Plasmid Clarification Plate into the lid. 3. Transfer 700–850 µl of lysate into each well of the Clarification Plate. 4. Apply vacuum for 1 minute, until the lysate completely passes through the Clarification Plate. Release the vacuum and discard the Clarification Plate. 5. Remove the Filter Plate from the manifold base and insert it into the manifold lid. 6. Apply vacuum for 1–2 minutes, until all the lysate passes through the Filter Plate. Release the vacuum. 7. *Optional* (*for EndA⁺ strains*): Add 600 µl Wash Buffer I with isopropanol into each well of the Filter Plate. Apply vacuum for 1 minute. Release the vacuum. 8. ***Required***: Add 900 µl Wash Buffer II with ethanol (see Before Starting) into each well of the Filter Plate. Apply vacuum for 1 minute. Release the vacuum. 9. Repeat Wash Buffer II (Step 8), one more time. 10. Apply vacuum for 10 minutes to remove any residual ethanol from the Filter Plate. Release the vacuum. 11. Place the PureLink™ Pro 96 Elution Plate into the manifold base. Replace the manifold lid on top and insert the Filter Plate into lid. 12. Add 50–175 µl Elution Buffer into each well of the Filter Plate. 13. Incubate at room temperature for 1 minute. 14. Apply vacuum for 2 minute to elute the DNA into the elution plate. Release the vacuum. 15. Seal the elution plate with Foil Tape (included with kit) and store plasmid DNA at –20ºC (long-term), or 4ºC (short-term), or proceed to the downstream application.

Kit Contents and Storage

Types of Products

This manual is supplied with the following products:

Product	Quantity	Catalog No.
PureLink™ Pro Quick96 Plasmid Purification Kit	4 × 96 preps	K2110-04A
	24 × 96 preps	K2110-24A

Shipping and Storage

All contents of the PureLink™ Pro Quick96 Plasmid Purification Kits are shipped at room temperature.

Upon receipt, store all contents at room temperature. Kit contents are stable at room temperature for up to six months.

Kit Contents

The contents of the PureLink™ Pro Quick96 Plasmid Purification Kits are described below.

Note: Some reagents provided in excess of the amount needed.

Box Contents	Quantity	
	K2110-04A	K2110-24A
PureLink™ Pro 96 Resuspension Buffer	200 mL	2 × 600 mL
PureLink™ Pro 96 Lysis Buffer	200 mL	2 × 600 mL
PureLink™ Pro 96 Neutralization Buffer	200 mL	2 × 600 mL
PureLink™ Pro 96 RNase A	4 mL	24 mL
PureLink™ Pro 96 Elution Buffer	100 mL	600 mL
PureLink™ 96 Well Plasmid Filter Plate	4 each	24 each
PureLink™ Pro 96 Plasmid Clarification Plate	4 each	24 each
PureLink™ Pro 96 Wash Buffer I	200 mL	1 L
PureLink™ Pro 96 Wash Buffer II (5X) (in 1 L bottles)	200 mL	2 × 600 mL
PureLink™ Pro 96 Elution Plate (part no. 466111)	4 each	---
PureLink™ Pro Elution Plate (part no. 466130)	---	24 each
PureLink™ Pro 96 Growth Block (Square-well)	4 each	24 each
PureLink™ Air Porous Tape	4 pieces	24 each
Foil Tape	4 pieces	24 each
PureLink™ Pro Quick96 Wash Buffer I - Dilution Bottle (1 L bottle)	---	1 each
PureLink™ Quick 96 Wash Buffer II - Dilution Bottle (1 L bottle)	---	1 each

Introduction

About the Kit

Kit Usage

The PureLink™ Pro Quick96 Plasmid Purification Kits are designed for rapid, high-throughput purification of up to 96 samples of plasmid DNA, isolated from *E. coli*.

The PureLink™ Pro Quick96 Plasmid Purification Kits combine advanced silica-membrane extraction with optimized 96-well plate designs to obtain consistent high yields of high-purity plasmid DNA using vacuum or centrifugation.

The PureLink™ Pro Quick96 Plasmid Purification Kits are designed for use in a vacuum manifold and are compatible with most automated liquid handling workstations (see page 7).

System Overview

Bacterial cells are grown in an antibiotic media (see page 10), and harvested by centrifugation. The cells are resuspended in Resuspension Buffer containing RNase A, and then lysed using an alkaline/SDS (sodium dodecyl sulfate) Lysis Buffer. Lysed cells are neutralized for binding with a Neutralization Buffer containing large amounts of chaotropic ions. Once neutralized, crude lysate is filtered through the PureLink™ Pro 96 Plasmid Clarification Plate to remove unwanted cellular debris. DNA in the cleared lysate is then reversibly bound to the silica membrane of the PureLink™ 96 Well Plasmid Filter Plate and cellular impurities are removed by washing. High-purity plasmid DNA is then eluted in PureLink™ Pro 96 Elution Buffer (10 mM Tris-HCl, pH 8.5).

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About the Kit, Continued

Advantages

- Rapid and efficient purification of plasmid DNA with consistent high yields and high-quality results.
- Simultaneous processing of up to 96 high-copy number plasmid DNA samples in 90 minutes or less.
- Ability to isolate up to 20 µg per well of purified plasmid DNA from 0.5 –3 mL of overnight bacterial cultures.
- Designed for use with a vacuum manifold for manual purification or with automated liquid handling systems.
- Improved plate design with nozzles that prohibit cross-contamination when properly assembled.

Downstream Applications

Plasmid DNA isolated using the PureLink™ Pro Quick96 Plasmid Purification Kits are suitable for a variety of downstream applications including:

- Automated fluorescent sequencing
- Cloning
- PCR
- Restriction enzyme digestion
- Screening

Product Specifications

Kit Specifications

Instrument Compatibility	Vacuum manifold or centrifuge (see page 4)
Processing Time:	90 min/96 preps or 45 min/48 preps
Binding Technology:	Silica membrane in a 96-well plate
Starting Material	3 mL lysate/well
Binding Capacity:	40 µg
Elution Volume:	75–175 µl (50–175 µl centrifuge)
DNA Yield:	5–20 µg (from 3 mL LB)
DNA Concentration:	50–200 ng/µl

Note: Processing time may vary depending on workstation configuration and number of samples processed. DNA yield may vary depending on a variety of factors (see page 5).

PureLink™ Pro 96 Plasmid Clarification Plate

Description:	Filters unwanted cellular debris from the lysate.
Dimensions:	Follows standard SBS (Society for Biomolecular Screening) footprint.
Volume:	1.5 mL

PureLink™ 96 Well Plasmid Filter Plate

Description:	Utilizes silica membrane extraction to reversibly bind DNA.
Dimensions:	Follows standard SBS (Society for Biomolecular Screening) footprint.
Volume:	1.5 mL
Binding Capacity:	40 µg

PureLink™ Pro 96 Elution Plate (part no. 466111)

Description:	Square-well block to collect purified DNA eluted from the Filter Plate.
Dimensions:	Follows standard SBS (Society for Biomolecular Screening) footprint.
Volume:	1.7 mL

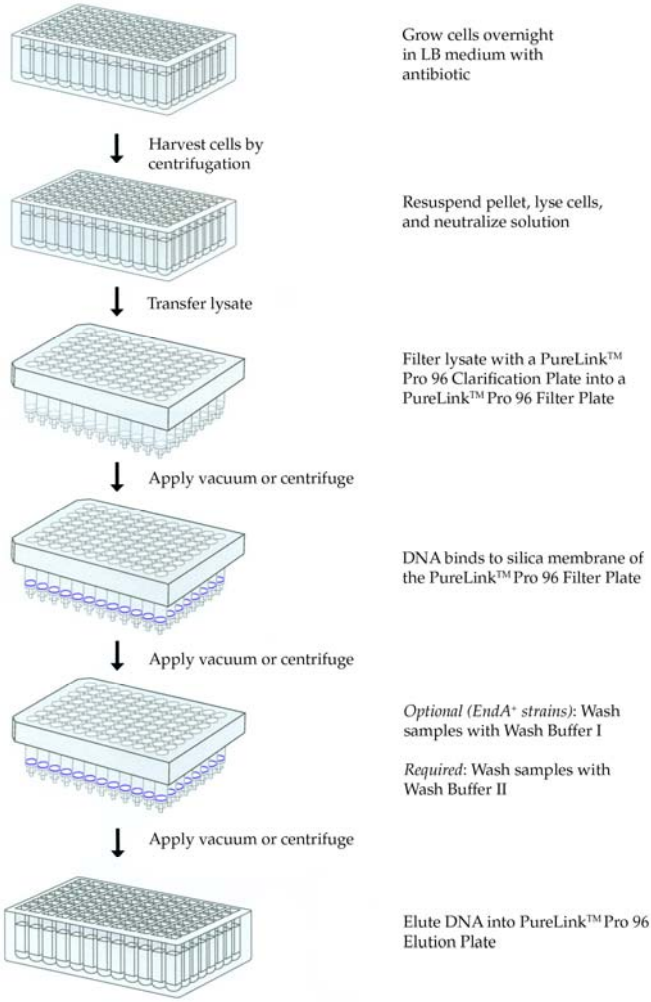
PureLink™ Pro Elution Plate (part no. 466130)

Description:	U-bottom microtiter plate to collect purified DNA eluted from the Filter Plate.
Dimensions:	Follows standard SBS (Society for Biomolecular Screening) footprint.
Volume:	300 µL

Experimental Overview

Workflow

The flow chart for purifying plasmid DNA with the PureLink™ Pro Quick96 Plasmid Purification Kit using plate-based centrifugation or vacuum for high-throughput processing of samples is shown below.



Methods

General Guidelines

The PureLink™ Neutralization Buffer and the PureLink™ Wash Buffer I contain guanidinium hydrochloride (an irritant). **Do not** add bleach or acidic solutions directly to solutions that contain guanidinium hydrochloride or sample preparation waste, as reactive compounds and toxic gases are formed.

The PureLink™ Lysis Buffer contains sodium hydroxide and SDS, which are considered to be irritants and hazardous.

For your protection, wear a laboratory coat, gloves and safety glasses when handling these buffers.

Plasmid DNA yield depends on a variety of factors:

- Plasmid copy number
- Plasmid size (< 15 kb)
- Type of growth medium used
- Bacterial host strain
- Bacterial culture growth
- Volume of elution buffer used

Follow the recommendations below for optimal results with plasmid DNA yield:

- Always use high-copy number plasmid (see page 10).
- Use the recommended medium with appropriate antibiotic and cell volume for plasmid isolation (see page 10).
- Always start the bacterial cell culture from a freshly streaked plate to avoid loss of the plasmid.

Continued on next page

General Guidelines, Continued

Recovery of Elution Volume

Plasmid DNA is eluted in 50–175 µl of PureLink™ Pro 96 Elution Buffer. Change the volume of elution buffer used to obtain your desired final concentration of DNA. For increased DNA yield, use a higher volume of elution buffer. For increased DNA concentration, use a lower volume of elution buffer.

A reduction in recovered volume relative to the starting volume is normal when eluting from plates.

The table below presents the typical volume of eluate recovered from the initial volume of elution buffer, when following the kit protocol.

Elution buffer volume	Recovered elution buffer (±5 µl)	
	Centrifuge	Vacuum
50 µl	35 µl	25 µl
75 µl	65 µl	50 µl
100 µl	90 µl	80 µl
125 µl	115 µl	95 µl
150 µl	140 µl	120 µl
175 µl	155 µl	145 µl

Continued on next page

General Guidelines, Continued

Processing Fewer than 96 Samples

You can use a portion of the PureLink™ Pro 96 Clarification Plate and Plasmid Filter Plate to isolate plasmid DNA from fewer than 96 samples. **Each well in the plates can only be used once**.

To process fewer than 96 samples:

1. Cover the entire surface of the Clarification Plate plate **or** Plasmid Filter Plate with Foil Tape (see page 27).
2. Just prior to filtering the lysate through the Clarification Plate, or binding the DNA to the Plasmid Filter Plate, score the foil around the wells to be used with a clean, sharp blade. Peel away the foil to expose the wells containing the samples.
3. Keep all unused wells in the plate sealed with Foil Tape during purification to obtain a uniform vacuum and avoid contaminating unused wells.

Important: When performing **Purification by Vacuum Manifold**, only the plate that is inserted into the vacuum manifold lid needs to be covered. **Do not** cover the bottom plate in the manifold base, or the nozzles of the top plate will not fit into the wells of the bottom plate, preventing a tight vacuum seal.

Instrument Compatibility

The PureLink™ 96 Well Plasmid Filter Plates are compatible with the following instruments:

- **Vacuum Manifold:** The manifold must accommodate the PureLink™ 96 Well Plates (half-skirted filter plate) and be capable of collecting the filtrate (e.g. EveryPrep™ Universal Vacuum Manifold from Invitrogen).
- **Centrifuge:** The centrifuge must be capable of centrifuging 96-well plates at ≥2,100 × g, and accommodate a 7.0 cm microtiter plate stack.
- **Automated Liquid Handling Workstation:** The workstation must be equipped with a vacuum manifold and a vacuum source, and accommodate the PureLink™ 96 Well Plate (half-skirted filter plate).

Before Starting

Introduction

Instructions for preparing the PureLink™ buffer solutions for plasmid DNA purification are provided below. Prepare the buffer solutions **before** beginning the purification.

Materials Needed

- 96% Ethanol
- *Optional:* 100% Isopropanol
- *Optional:* Water bath (37°C)

Preparing Resuspension Buffer with RNase A

Prepare the PureLink™ Pro 96 Resuspension Buffer with the PureLink™ Pro 96 RNase A as described below:

For 4 × 96 preps

1. Transfer the entire contents of the PureLink™ Pro 96 RNase A bottle into the Resuspension Buffer bottle. Mix thoroughly.
2. Mark the label on the Resuspension Buffer to indicate that RNase A has been added. Store at 4°C.

For 24 × 96 preps

1. Add 12 mL of PureLink™ Pro 96 RNase A into each Resuspension Buffer bottle. Mix thoroughly.
2. Mark the labels on the Resuspension Buffer to indicate that RNase A has been added. Store at 4°C.

Note: RNase A digestion is performed during sample preparation to degrade RNA present in the sample and to minimize RNA contamination in the purified DNA sample. RNA contamination can inflate the DNA content measured at 260 nm.

Lysis Buffer

Store the Lysis Buffer at room temperature (≥20°C).

The Lysis Buffer contains SDS, which can precipitate out of the buffer if stored below 20°C. If a precipitate is present, incubate the Lysis Buffer in a 37°C water bath for 5 minutes, or until the SDS redissolves and the solution clears.

Avoid shaking the Lysis Buffer, as this can lead to foaming.

Continued on next page

Preparing Wash Buffer II

Prepare Wash Buffer II as described below.

Each 1,000 mL Wash Buffer II prepared with ethanol provides enough buffer for approximately 550 reactions. Excess Wash Buffer II is provided for use with automated liquid handling systems.

For 4 × 96 preps

1. Add 800 mL 96% ethanol to 200 mL Wash Buffer II (5X) (provided in a 1 L bottle), and mix well.
2. Mark the label on the bottle to indicate that ethanol has been added. Store at room temperature.

For 24 × 96 preps

1. Transfer 200 mL of Wash Buffer II (5X) into the **reusable** dilution bottle (supplied with kit).
2. Add 800 mL of 96% ethanol to the dilution bottle containing the 200 mL of Wash Buffer II (5X), and mix well. Store at room temperature.

   Note: The dilution bottle is reusable. Clean the dilution bottle thoroughly after each use. **Do not** autoclave the dilution bottle.

Preparing Wash Buffer I

Wash Buffer I is an **optional** buffer, used in purification of plasmid DNA from EndA$^+$ strains of bacteria. Prepare Wash Buffer I as described below.

Each 60 mL Wash Buffer I prepared with isopropanol provides enough buffer for one 96 well plate.

For 4 × 96 preps

1. Add 133 mL 100% isopropanol to 200 mL Wash Buffer I (provided in a 500 mL bottle), and mix well.
2. Mark the label on the bottle to indicate that isopropanol has been added. Store at room temperature.

For 24 × 96 preps

1. Transfer 36 mL of Wash Buffer I into a clean bottle.
2. Add 24 mL of 100% isopropanol to the bottle containing the 36 mL of Wash Buffer I, and mix well.
3. Label the bottle containing Wash Buffer I with isopropanol. Store at room temperature.

Growing Cells and Preparing Lysates

Materials Needed

- Growth media (see **Recommended Media**, below)
- Appropriate antibiotic
- Centrifuge capable of ≥2,100 × g with buckets that can accommodate 96-well plates with a plate height clearance of 7.0 cm.
- Shaking incubator at 37°C (200–250 rpm is optimal)
- *Optional*: Culture flask
- PureLink™ Pro 96 Growth Block
- PureLink™ Pro 96 Resuspension Buffer containing RNase A (see page 8)
- PureLink™ Pro 96 Lysis Buffer
- PureLink™ Pro 96 Neutralization Buffer
- Gas-permeable Foil

Recommended Media

The PureLink™ Pro Quick96 Plasmid Purification Kits are designed to isolate plasmid DNA from >3 mL of *E. coli* cells grown overnight in a 96-well square block.

Use of **LB** (Luria-Bertani) **medium** is recommended for optimal growth of the *E. coli* bacterial cells.

Alternatively, rich media, such as TB (Terrific Broth) or 2X YT, may be used to grow the bacteria cells. If rich media is used, the volume of cells used for isolation may need to be reduced to avoid clogging the plates.

Note: Using rich media for growing bacteria causes the bacteria to grow faster and may lead to overgrown cultures with partially degraded or contaminated chromosomal DNA.

Recommended Cell Volume

The recommended cell volume for use with the PureLink™ Pro Quick96 Plasmid Purification Kits is 3 mL of cells/well.

Using cell volumes >3 mL may result in inefficient cell lysis or cross-contamination of samples.

Plasmid Copy Number

The PureLink™ Pro Quick96 Plasmid Purification Kits are designed to isolate and purify plasmid DNA from **high-copy** number plasmids.

LB medium is recommended for growing the high-copy number bacterial cells.

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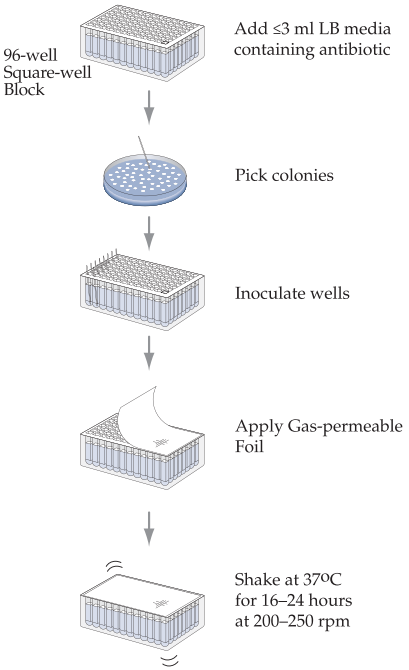
Growing Cells and Preparing Lysates,
Continued

Preparing 96-Well Cultures

Use the supplied PureLink™ Pro 96 Growth Block to prepare and grow the overnight *E. coli* cell cultures, as described below.



1. Add an appropriate concentration of antibiotic to LB medium.
2. Place ≤3 mL of the LB medium containing the antibiotic into each well of the Growth Block.

   Note: To avoid cross-contamination due to spilling during incubation, do not exceed a total culture volume of 3 mL.
3. Pick a single, well-isolated bacterial colony with a sterile toothpick (colonies should be no more than two weeks old). Drop the toothpick into a well of the Growth Block.
4. Repeat for all 96 wells of the Growth Block. Remove toothpicks and discard into a biowaste container.
5. Cover the Growth Block securely with the supplied gas-permeable foil.
6. Incubate the block containing the cultures in a 37ºC shaking incubator for 16–24 hours at 200–250 rpm.

   Note: Growth times for bacterial cultures of 16–24 hours are usually sufficient to produce optimal results. If, however, your bacterial cultures are growing poorly, increase the incubation time from 16–24 hours to 16–30 hours.
7. Proceed to **Harvesting Cells and Preparing Lysates** (page 13).

Continued on next page

Growing Cells and Preparing Lysates,
Continued

Preparing Growth Culture in Flask

Follow the steps below to grow *E. coli* cells in a culture flask:

1. Inoculate a single colony from a freshly streaked LB plate into 1–5 mL LB medium with appropriate antibiotic.
2. Grow cells at 37°C overnight on a shaking incubator (200–250 rpm is optimal).
3. Transfer the overnight culture to a 250 mL culture flask containing ~150 mL LB medium with appropriate antibiotic.
4. Grow cells at 37°C overnight on a shaking incubator at 200–250 rpm.
5. Proceed to **Harvesting Cells and Preparing Lysates**, page 13.

Important

- For harvesting the bacterial cells (next page), centrifuge the cells at 2,100 × g. Centrifugation at higher forces may produce tight pellets which are more difficult to resuspend.
- During lysis and neutralization (next page), perform all mixing steps **gently** to prevent shearing of chromosomal DNA and contamination of plasmid DNA with chromosomal DNA.
- If you are performing lysis on an automated liquid handling workstation, mix the suspension gently by shaking the block on a shaker. **Do not** vortex the lysate.

Continued on next page

Growing Cells and Preparing Lysates,
Continued

Harvesting Cells and Preparing Lysates

1. Harvest the cells as follows:

   Square-well Growth Block: Remove the sealed Growth Block from the shaking incubator and place it in the centrifuge rotor. Centrifuge at 2,100 × g for 10 minutes.

   Culture Flask: Remove the flask from the shaking incubator and transfer 1.5–3 mL of culture cells into each well of the PureLink™ Pro 96 Growth Block (supplied with kit). Seal the Growth Block with Foil Tape (page 27) and place the Growth Block in the centrifuge rotor. Centrifuge at 2,100 × g for 10 minutes.
2. Remove the Growth Block from the centrifuge. Discard the seal and remove the media from the wells.
3. Add 250 µl Resuspension Buffer with RNase A (page 8) into each well of the Growth Block. Shake the Growth Block gently until cell suspension is homogeneous. No cell clumps should be visible.
4. Add 250 µl Lysis Buffer to each well of the Growth Block. Shake the block **gently** to mix. **Do not** mix by pipetting up and down.
5. Incubate the lysate at room temperature for 2–5 minutes. **Do not exceed 5 minutes.**
6. Add 350 µl Neutralization Buffer into each well of the Growth Block. Mix by **gently** pipetting up and down.
7. Proceed to **Centrifuge Protocol** (page 14), **Vacuum Manifold Protocol** (page 18), or **EveryPrep™ Universal Vacuum Manifold Protocol** (page 28).

Purification Using Centrifugation

Introduction

Instructions are provided below to isolate plasmid DNA from overnight bacterial cultures using centrifugation.

Perform all purification steps at room temperature.

Materials Needed

- Centrifuge capable of reaching ≥2,100 × g with buckets that can accommodate 96-well plates with a plate height clearance of 7.0 cm.
- 96 Deep-well Block or equivalent (see page 27).

  Note: 96-well square-well blocks are required for the centrifugation steps and **must be purchased separately**.
- *Optional:* Sterile, distilled water (pH >7)
- PureLink™ Pro 96 Plasmid Clarification Plate
- PureLink™ 96 Well Plasmid Filter Plate
- PureLink™ Pro 96 Elution Plate
- *Optional*: PureLink™ Pro 96 Wash Buffer I prepared with isopropanol (see page 9)
- PureLink™ Pro 96 Wash buffer II prepared with ethanol (see page 9)
- PureLink™ Pro 96 Elution Buffer

Follow the recommendations below to improve plasmid DNA yield using centrifugation:

- Use the recommended centrifugation forces.
- Pipet the Elution Buffer or water into the center of the well for proper elution.
- Perform a 1 minute incubation with Elution Buffer.
- Increase the Elution Buffer volume up to 150 µl to increase DNA Yield (note that the DNA will be diluted).
- Decrease the Elution Buffer volume to 50–80 µl to increase the DNA concentration.
- If you are using water for elution, always use sterile, distilled water with pH >7.

Continued on next page

Purification Using Centrifugation, Continued

Note

Using a 2 mL 96 Deep-well Block allows the nozzles of the PureLink™ Pro 96 Plasmid Filter and Clarification Plates to be inserted into the Deep-well Block without contacting buffer flow-through during centrifugation.

Filtering Lysates and Binding DNA

1. Harvest the overnight bacterial cell culture, then resuspend and lyse the cells according to the protocol on page 13.
2. Stack a PureLink™ Pro 96 Plasmid Clarification Plate on top of a **new** 96 Deep-well Block. **Do not** use the Growth Block used to grow the overnight bacterial cell cultures.
3. Transfer the lysates from Step 1 (above) into the Clarification Plate with a multichannel pipettor.
4. Place the plate stack into the centrifuge and centrifuge for 2 minutes at 2,100 × g to filter the lysate into the wells of the Deep-well Block.
5. Remove the plate stack from the centrifuge and **discard** the Clarification Plate.
6. Stack a PureLink™ 96 Well Plasmid Filter Plate on top of a **new** 96 Deep-well Block.
7. Transfer the filtered lysates from the Deep-well Block in Step 4 into the wells of the Plasmid Filter Plate using a multichannel pipettor.
8. Place the plate stack into the centrifuge and centrifuge the stack for 2 minutes at 2,100 × g to bind the DNA to the membrane of the Plasmid Filter Plate.
9. Remove the plate stack from the centrifuge. Empty the flow-through from the Deep-well Block into an appropriate waste container.
10. Proceed to **Washing DNA** (next page).

Continued on next page

Purification Using Centrifugation, Continued

Note

The PureLink™ Pro Quick96 Plasmid Purification Kits contain two wash buffers, PureLink™ Pro 96 Wash Buffer I and PureLink™ Pro 96 Wash Buffer II.

Use of Wash Buffer I is **optional**, and is recommended when the bacterial host strain has high endogenous nuclease activity, or if you need to improve downstream results.

Washing DNA

1. Restack the Plasmid Filter Plate on top of the Deep-well-Block from the previous step (Step 9, page 15).
2. *Optional:* Add 600 µl Wash Buffer I with isopropanol to each well of the Plasmid Filter Plate.
3. Place the plate stack into the centrifuge and centrifuge for 2 minutes at 2,100 × g.
4. Remove the plate stack and empty the flow-through from the Deep-well Block into an appropriate waste container. Restack the Plasmid Filter Plate on top of the Deep-well Block.
5. *Required*: Add 900 µl of prepared Wash Buffer II (see page 9) into each well of the Plasmid Filter Plate.
6. Place the plate stack into the centrifuge and centrifuge for 2 minutes at 2,100 × g.
7. **Repeat Wash Buffer II (Steps 5–6), one more time**.
8. Remove the plate stack from the centrifuge and empty the flow-through from the Deep-well Block into an appropriate waste container.
9. Restack the Plasmid Filter Plate on top of the Deep-well Block and centrifuge the plate stack for 10 minutes at 2,100 × g.
10. Remove the plate stack from the centrifuge. Empty the flow-through into an appropriate waste container and **discard** the square-well block.
11. Place the Plasmid Filter Plate with the filter side down on a stack of paper towels, and pat firmly to blot any residual liquid.
12. Proceed to **Eluting DNA** (next page).

Continued on next page

Purification Using Centrifugation, Continued

Eluting DNA

1. Stack the Plasmid Filter Plate on top of a **new** PureLink™ Pro 96 Elution Plate.
2. Add 50–175 µl Elution Buffer to the center of each well of the Plasmid Filter Plate.

   Note: If higher concentrations of DNA are required, reduce the volume of Elution Buffer used to 50–75 µl. To achieve higher DNA yield, use a higher volume of Elution Buffer (see **Recovery of Elution Volume** on page 6 for details).
3. Incubate samples in elution buffer for 1 minute.
4. Place the plate stack into the centrifuge and centrifuge for 2 minutes at 2,100 × g to elute the DNA from the Plasmid Filter Plate into the Deep-well Block.
5. Remove the plate stack from the centrifuge. **The eluted DNA is in the Elution Plate.**
6. Seal the Elution Plate containing the plasmid DNA with Foil Tape (supplied with kit). Store eluted DNA at –20°C (long-term) or 4°C (short-term), or proceed to the downstream application.

Purification Using a Vacuum Manifold

Introduction

Instructions are provided below to manually isolate plasmid DNA from overnight bacterial cultures, using a vacuum manifold. The compatibility of the PureLink™ Pro 96 plates with various automated instruments is described on page 4.

Perform all purification steps at room temperature. For a protocol using the EveryPrep™ Universal Vacuum Manifold, see page 28.

Materials Needed

- Vacuum manifold and vacuum pump (producing pressure of 12–15 in. Hg) or automated liquid handling system
- *Optional*: Centrifuge capable of reaching ≥2,100 × g with buckets that can accommodate 96-well plates with a plate height clearance of 7.0 cm.
- *Optional*: Sterile, distilled water (pH >7)
- PureLink™ 96 Well Plasmid Filter Plate
- PureLink™ Pro 96 Plasmid Clarification Plate
- 96 Deep-well Block (see page 27)
- PureLink™ Pro 96 Elution Plate
- *Optional*: PureLink™ Pro 96 Wash Buffer I prepared with isopropanol (see page 9)
- PureLink™ Pro 96 Wash buffer II prepared with ethanol (see page 9)
- PureLink™ Pro 96 Elution Buffer

Follow the recommendations below to improve DNA yield.

- Use the recommended vacuum pressure.
- Pipette the elution buffer or water into the center of the well for proper elution.
- Perform a 1 minute incubation with elution buffer.
- Increase elution buffer volume up to 175 µl to increase DNA Yield (note that the DNA will be diluted).
- Decrease elution buffer volume to 75–80 µl to increase DNA concentration.
- If you are using water for elution, always use sterile water with pH >7.

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Purification Using a Vacuum Manifold,
Continued

Calibrating Vacuum for Use with 96-Well Plates

Use a vacuum pressure of –12 to –15 in. Hg, or reduce the vacuum pressure until a flow rate of 1–2 drops per second is achieved to obtain the best results.

Using higher vacuum pressure than the recommended pressure may cause sample splattering or inefficient DNA binding, while using lower vacuum pressure will affect the elution resulting in lower recovery.

To check the vacuum pressure:

1. Place an unused PureLink™ 96 Well Plasmid Filter Plate on top of the vacuum manifold. Seal the plate with Foil Tape.
2. Apply vacuum and check the vacuum pressure on the vacuum regulator (usually attached to the manifold or a vacuum pump).
3. Adjust the vacuum pressure on the regulator to obtain the recommended pressure of –12 to –15 in. Hg.

Note: During purification the vacuum pressure may exceed the recommended value.

Continued on next page

Purification Using a Vacuum Manifold,
Continued

Filtering Lysate and Binding DNA

1. Assemble the vacuum manifold according to the manufacturer's specifications.

   If using an automated liquid handling system, prepare the workstation according to the manufacturer's specifications.

2. Place a PureLink™ 96 Well Plasmid Filter Plate inside the vacuum manifold base and place the manifold lid on top.
3. Insert a PureLink™ Pro 96 Plasmid Clarification Plate onto the manifold lid. Align the Clarification Plate with the Plasmid Filter Plate.

   Note: If processing fewer than 96 samples, cover unused plate wells with Foil Tape (see page 7).

4. Transfer the lysate (see page 13) to the Clarification Plate using a multichannel pipettor or robotic loading device.
5. Apply vacuum for 2 minutes until all lysate passes through the Clarification Plate.
6. Release the vacuum and **discard** the Clarification Plate.
7. Remove the manifold lid and take out the Plasmid Filter Plate. Replace the manifold lid and place the Plasmid Filter Plate onto the manifold lid.
8. Apply vacuum for 1 minute to bind the DNA from the lysate to the membrane of the Plasmid Filter Plate.
9. Release the vacuum.
10. Proceed to **Washing DNA** (next page).

Continued on next page

Purification Using a Vacuum Manifold,
Continued

Note

The PureLink™ Pro Quick96 Plasmid Purification Kits contain two wash buffers, PureLink™ Pro 96 Wash Buffer I and PureLink™ Pro 96 Wash Buffer II.

Use of Wash Buffer I is **optional**, and is recommended when the bacterial host strain has high endogenous nuclease activity, or if you need to improve downstream results.

Washing DNA

1. After binding the DNA (page 20), remove the Plasmid Filter Plate and the manifold lid. Place a waste tray into the manifold base to collect the flow-through, if necessary. Replace the manifold lid and insert the Plasmid Filter Plate onto the manifold lid.
2. **Optional:** Add 600 µl Wash Buffer I with isopropanol into each well of the Plasmid Filter Plate.
3. Apply vacuum for 1 minute. Release the vacuum.
4. Add 900 µl of prepared Wash Buffer II with ethanol (page 9) into each well of the Plasmid Filter Plate.
5. Apply vacuum for 1 minute. Release the vacuum.
6. **Repeat Wash Buffer II (Steps 4–5), one more time.**
7. Proceed to Step 8 if you are continuing the purification process using vacuum, otherwise proceed to **Eluting DNA with Centrifugation** (page 23).
8. Apply vacuum for 10 minutes. Release the vacuum.
9. Place the Plasmid Filter Plate with the filter side down on a stack of paper towels, and pat firmly to blot any residual liquid.
10. Proceed to **Eluting DNA Using Vacuum** (next page).

Continued on next page

Purification Using a Vacuum Manifold,
Continued

Note

If higher concentrations of final DNA are required, elute the plasmid by centrifugation. Complete all purification steps with vacuum through the second wash step, then proceed to **Eluting DNA with Centrifugation** (page 23).

Eluting DNA Using Vacuum

1. After washing the samples and drying the Plasmid Filter Plate, remove the manifold lid and the Plasmid Filter Plate from the manifold base.
2. Remove the waste tray from the manifold base.
3. Place a PureLink™ Pro 96 Elution Plate into the manifold base and replace the manifold lid. Insert the Plasmid Filter Plate onto the lid and align the Plasmid Filter Plate with the Elution Plate.
4. Add 75–175 µl Elution Buffer (10 mM Tris-HCl, pH 8.5) into the center of each well of the Plasmid Filter Plate.

   ***Optional*:** Elute with 75–175 µl of sterile, distilled water (pH >7).
5. Incubate at room temperature for 1 minute.
6. Apply vacuum for 2 minute to elute the DNA from the Plasmid Filter Plate into the Elution Plate.
7. Release the vacuum and remove the Elution Plate from the manifold base.
8. Seal the Elution Plate with Foil Tape (supplied with kit). Store the eluted DNA at –20ºC (long-term), or 4ºC (short-term), or proceed to downstream application.

Continued on next page

Purification Process Using Vacuum,
Continued

Important

Additional 2 mL Deep-well Blocks are required for eluting DNA with centrifugation and **must be purchased separately**.

Eluting DNA with Centrifugation

1. Remove the Plasmid Filter Plate from the vacuum manifold lid and gently tap the nozzles on the bottom of the plate on paper sheets.
2. Stack the Plasmid Filter Plate on top of a **new** 2 mL 96 Deep-well Block (page 27). **Do not** use the Growth Block used to grow overnight bacterial cultures.
3. Place the plate stack inside a centrifuge. Centrifuge for 10 minutes at 2,100 × g to dry the membrane of the Plasmid Filter Plate.
4. Remove the plate stack from the centrifuge. Empty the flow-through into an appropriate waste container and **discard** the Deep-well Block.
5. Stack the Plasmid Filter Plate on a **new** 2 mL Deep-well Block.

   Note: For eluting DNA with centrifugation, the elution volume used is significantly reduced. A 1 mL square-well block can be used for the elution step in place of a 2 mL square-well block.
6. Add 50–175 µl Elution Buffer directly onto the membrane of each well in the Plasmid Filter Plate.

   Note: Use 50–75 µl of Elution Buffer for eluting samples in a concentrated volume.
7. Incubate samples in elution buffer at room temperature for 1 minute.
8. Place the plate stack into the centrifuge and centrifuge for 2 minutes at 2,100 × g to elute the DNA from the Plasmid Filter Plate into the Elution Plate.
9. Remove the plate stack from the centrifuge and seal the Elution Plate containing the plasmid DNA with Foil Tape (supplied with kit).
10. Store the eluted DNA at –20°C (long-term), or 4°C (short-term), or proceed to down stream application.

Analyzing DNA Yield and Quality

Estimating DNA Yield

Plasmid DNA isolated using the PureLink™ Pro Quick96 Plasmid Purification Kits is easily quantitated using UV absorbance at 260 nm or using Quant-iT™ DNA Assay Kits (see page 27).

UV Absorbance

1. Measure the A_{260} of the solution using a spectrophotometer blanked against 10 mM Tris-HCl, pH 8.5.

   Note: Uou can use a microplate spectrophotometer with a path check sensor using a UV transparent 96-well plate for reading the UV absorbance. The path check sensor automatically normalizes the absorbance reading to a 1 cm path length.

2. Calculate the amount of DNA using the following formula:

DNA (µg) = A_{260} × 50 µg/mL solution measured in a cuvette with an optical path length of 1 cm.

Quant-iT™ DNA Assay Kits

The Quant-iT™ DNA Assay Kits provide a rapid, sensitive, and specific method for DNA quantitation with minimal interference from RNA, protein, or other common contaminants that affect UV absorbance readings.

The Quant-iT™ kits contain a state-of-the-art quantitation reagent, pre-diluted standards for standard curve, and a ready-to-use buffer. The assay is performed in a microtiter plate format and is designed for reading in standard fluorescent microplate readers. Follow manufacturer's recommendations to perform the assay.

Estimating DNA Quality

Typically, DNA isolated using the PureLink™ Pro Quick96 Plasmid Purification Kits have an A_{260}/A_{280} ≥1.70–1.80 when samples are diluted in Tris-HCl (pH 8.5). An A_{260}/A_{280} of >1.80 indicates that the DNA is reasonably clean of proteins that could interfere with downstream applications.

Absence of contaminating DNA and RNA may be confirmed by agarose gel electrophoresis.

Troubleshooting

Introduction

The table below describes solutions to possible problems you may experience with the PureLink™ Pro Quick96 Plasmid Purification Kits. For additional assistance, contact **Technical Support** (see page 28).

Problem	Cause	Solution
Incomplete lysis of bacterial cells	Too many cells used	• Reduce bacterial culture volume to 3 mL. • Use LB growth medium. Rich medium (*e.g.*, TB) can cause high cell densities.
	Cell pellet not properly resuspended	Increase the number of mixing cycles or the duration of the shaking. **Note:** No cell clumps should be visible prior to adding the lysis buffer.
	Precipitation of SDS	• Store the Lysis Buffer at room temperature (≥20°C). • Incubate the Lysis Buffer with the precipitate at 37°C for 5 minutes. Mix thoroughly before using.
Low plasmid yield	Incomplete lysis of bacterial cells	See above.
	High-copy number plasmid not used	Use high-copy number plasmid (see page 10).
	Not enough antibiotic used during bacterial cell cultivation	• Add the appropriate amount of antibiotic to the medium. • Ensure fresh stocks of medium with antibiotic are used.
	Bacterial cultures too old	• Use fresh cell cultures (see page 10).

Continued on next page

Troubleshooting, Continued

Problem	Cause	Solution
Low plasmid yield, continued	Ethanol not added to, or evaporated from, Wash Buffer II	• Add the appropriate amount of ethanol to the Wash Buffer II (see page 9). • Ensure the lid on the buffer is tightly closed.
	Poor elution conditions	• Use PureLink™ Pro 96 Elution Buffer, (10 mM Tris-HCl, pH 8.5). • If using nuclease-free water for elution, ensure pH values are pH >7.0. **Note:** Elution efficiencies drop strongly with buffers <pH 7.0.
Chromosomal DNA contamination	Cell culture volume too high	Reduce the cell culture volume if the lysate is too viscous for complete mixing.
	Overgrown bacterial cultures	See page 10 for procedures on growing bacterial cultures. **Note:** Overgrown cultures contain lysed cells and degraded DNA.
	Lysis too long	Lysis must not exceed 5 minutes.
	Excessive mixing after Lysis and Neutralization Buffer additions or before transfer of crude lysate to the clarifiction plate.	• Reduce the number of mixing cycles. • Decrease the shaker speed.
RNA present in the eluate	RNA was not completely degraded	• Ensure the RNase A is added to the Resuspension Buffer **before** use. • Reduce culture volume (if necessary).

Appendix

Additional Products

Additional Products

The following products are also available from Invitrogen: For more details on these products, visit our website at www.invitrogen.com or contact **Technical Support** (see page 28).

Product	Quantity	Catalog No.
EveryPrep™ Universal Vacuum Manifold	1 manifold	K2111-01
PureLink™ Foil Tape	50 each	12261-012
PureLink™ Air Porous Tape	50 each	12262-010
PureLink™ Quick Plasmid Miniprep Kit	50 preps	K2100-10
PureLink™ 96 HQ Mini Plasmid DNA Purification Kit	4 × 96 preps	K2100-96
Quant-iT™ Broad-Range DNA Assay Kit	1000 assays	Q-33130
Quant-iT™ PicoGreen® dsDNA Assay Kit *2000 Assays*	1 kit, 1 mL	P7589
Quant-iT™ PicoGreen® dsDNA Assay Kit *2000 Assays* *10 × 100 µl*	1 kit, 10 × 100 µl	P11496
96 Deep-well Blocks (square-well)	50 each	CS15196
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 in different agarose percentages and well formats, designed for fast, convenient electrophoresis of DNA samples.

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

For more details on these products, visit our website at www.invitrogen.com or contact **Technical Support** (see page 28).

Continued on next page

Purification Using the EveryPrep™ Universal Vacuum Manifold

Introduction

Instructions are provided below to purify DNA using the EveryPrep™ Universal Vacuum Manifold (see page 27). Refer to the manual for the EveryPrep™ Universal Vacuum Manifold for detailed instructions on operation with the 96 Well Top Plate. All steps are performed at room temperature.

Materials Needed

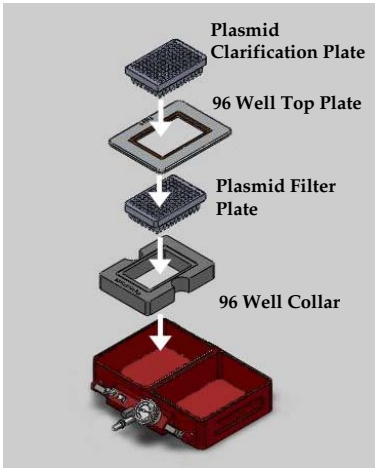
- Lysate from bacterial samples (see previous page)
- Vacuum manifold and vacuum pump (producing pressure of 12–15 in. Hg)
- PureLink™ Pro 96 Wash Buffer II with ethanol (page 9)
- PureLink™ Pro 96 Elution Buffer (supplied with kit)
- *Optional*: Centrifuge capable of ≥2,100 × g with buckets that can accommodate 96-well plates with a plate height clearance of 7.0 cm.
- *Optional:* Sterile, distilled water (pH >7)
- *Optional*: PureLink™ Pro 96 Wash Buffer I prepared with isopropanol (see page 9)
- PureLink™ 96 Well Plasmid Filter Plate (supplied with kit)
- PureLink™ Pro 96 Elution Plate (supplied with kit)
- Foil tape (supplied with kit)

Continued on next page

Purification Using the EveryPrep™ Universal Vacuum Manifold, Continued

EveryPrep™ Universal Vacuum Manifold Assembly

1. Place the 96 Well Collar in the Binding Chamber, seat the PureLink™ Pro 96 Well Filter Plate above the 96 Well Collar, cover the chamber with the 96 Well Top Plate, and place the PureLink™ Pro 96 Clarification Plate onto the Top Plate.



2. Proceed to **Binding DNA**, next page.

Note

The PureLink™ Pro Quick96 Plasmid Purification Kits contain two wash buffers, PureLink™ Pro 96 Wash Buffer I and PureLink™ Pro 96 Wash Buffer II.

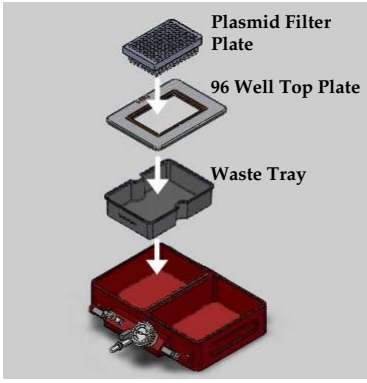
Use of Wash Buffer I is **optional**, and is recommended when the bacterial host strain has high endogenous nuclease activity, or if you need to improve downstream results.

Continued on next page

Purification Using the EveryPrep™ Universal Vacuum Manifold, Continued

Binding and Washing DNA

1. Transfer 700–850 µl of lysate from each well of the Growth Block to the corresponding position in the Clarification Plate. Cover unused wells with Foil Tape.
2. Apply vacuum for 1–2 minutes. Release the vacuum.
3. Discard the Clarification Plate and remove the Plasmid Filter Plate and 96 Well Collar from the binding chamber.
4. Place the Waste Tray in the Binding Chamber, cover the chamber with the 96 Well Top Plate, and place the Plasmid Filter Plate onto the Top Plate.



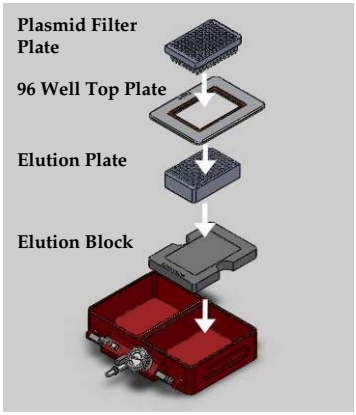
5. Apply vacuum for 1–2 minutes. Release the vacuum.
6. ***Optional (for EndA⁺ strains)*:** Add 600 µl of Wash Buffer I with isopropanol into each well of the Plasmid Filter Plate. Apply vacuum for 1 minute. Release the vacuum.
7. Add 900 µl of Wash Buffer II with ethanol (see page 9) into each well of the Plasmid Filter Plate.
8. Apply vacuum for 1 minute. Release the vacuum.
9. Repeat Steps 7 and 8 above.
10. Apply vacuum for an additional 10 minutes. Release the vacuum.
11. Place the Plasmid Filter Plate with the filter side down on a stack of paper towels, and pat firmly to blot any residual liquid.
12. Proceed to **Eluting DNA**, next page.

Continued on next page

Purification Using the EveryPrep™ Universal Vacuum Manifold, Continued

Eluting DNA

1. Place the Elution Block and a clean PureLink™ Pro 96 Elution Plate in the Elution Chamber, cover the top with the 96 Well Top Plate, and place the PureLink™ 96 Well Plasmid Filter Plate over the Top Plate.



2. Add 75–175 µl of PureLink™ Pro 96 Elution Buffer (10 mM Tris-HCl, pH 8.5) to the center of the membrane in each well of the Plasmid Filter Plate and incubate for 1 minute at room temperature (see page 6 for elution parameters).

   ***Optional*:** Elute with 75–175 µl of sterile, distilled water (pH >7).

3. Apply vacuum for 2 minutes. Release the vacuum. The plasmid DNA is eluted into the Elution Plate.
4. Use the plasmid DNA for the desired downstream application. To store the purified plasmid DNA, cover the wells with Foil Tape, and store at –20°C (long term), or 4°C (short term).

Note

If higher concentrations of final DNA are required, elute the plasmid by centrifugation. Complete all purification steps with vacuum through the second wash step, then proceed to **Eluting DNA with Centrifugation** (page 23).

Technical Support

World Wide Web

Visit the Invitrogen website at **www.invitrogen.com** for:

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

Contact Us

For more information or technical assistance, call, write, fax, or email. Additional international offices are listed on our website (www.invitrogen.com).

Corporate Headquarters:
5791 Van Allen Way
Carlsbad, CA 92008 USA
Tel: 1 760 603 7200
Tel (Toll Free): 1 800 955 6288
Fax: 1 760 602 6500
E-mail: tech_support@invitrogen.com

Japanese Headquarters:
LOOP-X Bldg. 6F
3-9-15, Kaigan
Minato-ku, Tokyo 108-0022
Tel: 81 3 5730 6509
Fax: 81 3 5730 6519
E-mail: jpinfo@invitrogen.com

European Headquarters:
Inchinnan Business Park
3 Fountain Drive
Paisley PA4 9RF, UK
Tel: +44 (0) 141 814 6100
Tech Fax: +44 (0) 141 814 6117
E-mail: eurotech@invitrogen.com

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Purchaser Notification, Continued

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invitrogen™

Corporate Headquarters
Invitrogen Corporation
5791 Van Allen Way
Carlsbad, CA 92008
T: 1 760 603 7200
F: 1 760 602 6500
E: tech_support@invitrogen.com

For country-specific contact information, visit our web site at **www.invitrogen.com**