invitrogen™

PureLink™ Quick96 Plasmid Kit

For high-throughput purification of Plasmid DNA

Catalog nos. K2110-04, K2110-24

Version B
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25-1013

User Manual

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Experienced Users Protocol

Introduction

This quick reference sheet is provided for experienced users of the PureLink™ Quick96 Plasmid Kits. If you are a first time user of these kits, refer to the details provided in this manual.

Note: If you will be performing all purification steps using centrifugation, refer to the support protocol for centrifugation located in the **Appendix** (page 25).

Step	Action
Grow Cells	Grow cells in a PureLink™ Quick96 Square-well Block or a culture flask using LB medium with appropriate antibiotic. Grow bacterial cell cultures overnight in a 37ºC shaking incubator at 200-250 rpms.
Harvest Cells and Prepare Lysates	1. Harvest cells by centrifugation at 1,000 × g for 10 minutes. Remove growth media. 2. Resuspend cell pellets in 250 µl Resuspension Buffer with RNase A (see page 10). 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. 5. Add 350 µl Neutralization Buffer to each well. Mix by **gently** pipetting up and down.
Purification Protocol Using Vacuum	**Before beginning:** An option is presented in this protocol, after the second wash step (Step 10, next page), to elute the final DNA using centrifugation (page 23). Use this option if you wish to obtain higher concentrations of final DNA. All steps prior to elution using centrifugation need to be completed using vacuum. 1. Assemble the vacuum manifold. Use a vacuum pressure of –200 to –400 millibars (mbars) 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™ Quick96 Plasmid Binding Plate inside the vacuum manifold base. Place the manifold lid on top and insert a PureLink™ Quick96 Plasmid Filter Plate into the lid. 3. Transfer 1.2-1.5 ml of lysate into each well of the filter plate. 4. Apply vacuum for 1-5 minutes until all lysate passes through the filter plate. Release vacuum and discard the filter plate. 5. Remove the binding plate from the manifold base and insert it into the manifold lid.

Continued on next page

Step	Action
Purification Protocol Using Vacuum, Continued	6. Bind the DNA to the binding plate by applying vacuum for 1 minute. Release vacuum. Remove the binding plate and lid from the manifold base. 7. Place the PureLink™ Quick96 Wash Plate into the manifold base. Replace manifold lid on top and insert the binding plate into the lid. 8. *Optional:* Add 600 µl Wash Buffer I (page 21) into each well of the binding plate. Apply vacuum for 1 minute. Release vacuum. 9. *Required:* Add 900 µl Wash Buffer II with ethanol (page 11) into each well of the binding plate. Apply vacuum for 1 minute. Release vacuum. 10. **Repeat** Wash Buffer II (Step 9), one more time. **Note:** If higher concentrations of final DNA are desired, proceed to **Eluting DNA with Centrifugation** (page 23), after completing Step 10. 11. Remove the wash plate and the waste tray from the manifold base. Replace the manifold lid on top of the base and insert the binding plate into the lid. 12. Apply vacuum for 10 minutes to remove any residual ethanol from the binding plate. Release vacuum. 13. *Optional:* Remove the binding plate and tap the nozzles on the bottom of the plate on paper sheets (included with kit) to remove any residual ethanol from the nozzles. 14. Place the PureLink™ Quick96 Elution Plate into the manifold base. Replace the manifold lid on top and insert the binding plate into lid. 15. Add 75-150 µl Elution Buffer into each well of the binding plate. 16. Incubate at room temperature for 1-3 minutes. 17. Apply vacuum for 1 minute to elute the DNA into the elution plate. Release vacuum. 18. Seal the elution plate with adhesive cover foil (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™ Quick96 Purification Kit	4 x 96 preps	K2110-04
	24 x 96 preps	K2110-24

Shipping and Storage

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

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

***After addition of RNase A to the Resuspension Buffer, store the buffer at 4ºC. The Resuspension Buffer containing RNase A is stable at 4ºC up to six months.**

Kit Contents K2110-04

The PureLink™ Quick96 Plasmid Kit contents (4 x 96 preps) are shipped in two separate boxes, as described below.

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

Box 1 of 2 Contents	Quantity
PureLink™ Quick96 Resuspension Buffer	200 ml
PureLink™ Quick96 Lysis Buffer	200 ml
PureLink™ Quick96 Neutralization Buffer	200 ml
PureLink™ Quick96 RNase A (lyophilized)	80 mg
PureLink™ Quick96 Elution Buffer	100 ml
PureLink™ Quick96 Plasmid Filter Plate	4 each
PureLink™ Quick96 Plasmid Binding Plate	4 each
PureLink™ Quick96 Wash Plate (includes six paper sheets/plate)	4 each

Box 2 of 2 Contents	Quantity
PureLink™ Quick96 Wash Buffer I	1000 ml
PureLink™ Quick96 Wash Buffer II	2 x 200 ml (in 1 L bottles)
PureLink™ Quick96 Elution Plate (includes one adhesive cover foil/plate)	4 each
PureLink™ Quick96 Square-well Block (includes one gas-permeable foil/plate)	4 each

Continued on next page

Kit Contents K2110-24

The contents of the PureLink™ Quick96 Plasmid Kit (24 x 96 preps) are shipped in six separate boxes, as described below.

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

Box 1 of 6 Contents	Quantity
PureLink™ Quick96 Resuspension Buffer	1000 ml
PureLink™ Quick96 Lysis Buffer	1000 ml
PureLink™ Quick96 Neutralization Buffer	1000 ml
PureLink™ Quick96 RNase A (lyophilized)	5 x 80 mg
PureLink™ Quick96 Elution Buffer	500 ml

Box 2 of 6 Contents	Quantity
PureLink™ Quick96 Wash Buffer I	4 x 1000 ml

Box 3 of 6 Contents	Quantity
PureLink™ Quick96 Plasmid Filter Plate	24 each
PureLink™ Quick96 Wash Plate (includes six paper sheets/plate)	12 each

Box 4 of 6 Contents	Quantity
PureLink™ Quick96 Plasmid Binding Plate	24 each
PureLink™ Quick96 Wash Plate (includes six paper sheets/plate)	12 each

Box 5 of 6 Contents	Quantity
PureLink™ Quick96 Square-well Block (includes one gas-permeable foil/plate)	24 each
PureLink™ Quick96 Elution Plate (includes one adhesive cover foil/plate)	24 each

Box 6 of 6 Contents	Quantity
PureLink™ Quick96 Wash Buffer I	1 x 1000 ml
PureLink™ Quick96 Wash Buffer II (concentrate)	2 x 1000 ml
PureLink™ Quick96 Wash Buffer II - Dilution Bottle	1 each

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 32).

Product	Quantity	Catalog No.
PureLink™ Vacuum Manifold	1 each	K2110-01
PureLink™ Vacuum Regulator	1 each	K2110-02
PureLink™ Adapter Frame (for automated purification)	1 each	K2110-03
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 x 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 x 100µl*	1 kit, 10 x 100 µl	P11496
96 Deep-well Blocks (square-well)	50 each	CS15196
Luria Broth Base (Miller's LB Broth Base)®, powder	500 g 2.5 kg	12795-027 12795-084
Ampicillin Sodium Salt, irradiated	200 mg	11593--027
Carbenicillin, Disodium Salt	5 g	10177-012

E-Gel® Agarose Gels and DNA Ladders

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

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** (page 32).

Introduction

Overview

Introduction

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

The Quick96 Plasmid 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 Quick96 Kits are designed to be used in a vacuum manifold and are compatible with certain automated liquid handling systems (see page 8). If high concentrations of final DNA are required, an optional protocol is provided to elute the DNA with centrifugation, using a reduced elution buffer volume (see page 23).

System Overview

Bacterial cells are grown in an antibiotic media (see page 12), and harvested by centrifugation. The cells are resuspended in Resuspension Buffer containing RNase, 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 vacuum filtered through the PureLink™ Quick96 Plasmid Filter Plate to remove unwanted cellular debris. DNA in the cleared lysate is then reversibly bound to the silica membrane of the PureLink™ Quick96 Plasmid Binding Plate and cellular impurities are removed by washing. High-purity plasmid DNA is then eluted in Elution Buffer (5 mM Tris-HCl, pH 8.5).

Continued on next page

Advantages of the Kit

The PureLink™ Quick96 Plasmid Kits offer the following 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 15 µg per well of purified plasmid DNA from 1.5 -5 ml of overnight bacterial cultures.
- Designed to be used 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™ Quick96 Plasmid Kits is suitable for a variety of downstream applications including:

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

Kit Specifications

Instrument Compatibility	Vacuum manifold or centrifuge (see page 8)
Processing Time:	90 min/96 preps or 45 min/48 preps
Binding Technology:	Silica membrane in a 96-well plate
Starting Material	1.2-1.5 ml lysate/well
Binding Capacity:	20 µg
Elution Volume:	75-150 µl
DNA Yield:	5-15 µg (from up to 5 ml LB, 3 ml TB, or 3 ml 2X YT)
DNA Concentration:	50-200 ng/µl

Note: Processing time may vary depending on workstation configuration and number of samples being processed. DNA yield may vary depending on a variety of factors (see recommendations on page 4).

Continued on next page

PureLink™ Quick96 Plasmid Filter Plate	Description:	The PureLink™ Quick96 Plasmid Filter Plate filters unwanted cellular debris from the lysate.
	Dimensions:	Following standard SBS (Society for Biomolecules Screening) footprint.
	Volume:	1.5 ml
PureLink™ Quick96 Plasmid Binding Plate	Description:	The PureLink™ Quick96 Plasmid Binding Plate uses silica membrane extraction to reversibly bind the DNA.
	Dimensions:	Following standard SBS (Society for Biomolecules Screening) footprint.
	Volume:	1.5 ml
	Binding Capacity:	20 µg
PureLink™ Quick96 Wash Plate	Description:	The PureLink™ Quick96 Wash Plate is a microtiter plate that is open on both sides, allowing the free flow of wash buffers. During washing, the nozzles of the binding plate protrude into the wells of the wash plate, preventing wash buffer from spraying onto the binding plate and reducing cross-contamination and ethanol carry-over.
	Dimensions:	Following standard SBS (Society for Biomolecules Screening) footprint.
	Volume:	N/A (plate is open on both ends).
PureLink™ Quick96 Elution Plate	Description:	The PureLink™ Quick96 Elution Plate is a U-bottom microtiter plate that collects purified DNA from the binding plate during elution.
	Dimensions:	Following standard SBS (Society for Biomolecules Screening) footprint.
	Volume:	300 µl

Methods

General Guidelines

Introduction

General guidelines for using the PureLink™ Quick96 Plasmid Kits are described below. To obtain high-quality plasmid DNA, review this section before starting the purification process.

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 13).
- Use the recommended medium with appropriate antibiotic and cell volume for plasmid isolation (see page 12).
- Always start the bacterial cell culture from a freshly streaked plate to avoid loss of the plasmid.

Continued on next page

Elution Parameters

Elution Buffer

Plasmid DNA is eluted using the PureLink™ Quick96 Elution Buffer, (5 mM Tris-HCl, pH 8.5). Sterile water (pH 8.0-8.5) can be substituted if the Tris-HCl in the elution buffer inhibits downstream reactions.

Elution Buffer Volume

Plasmid DNA is eluted in 75-150 µl of PureLink™ Quick96 Elution Buffer. You can 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. Use the graph below to determine the most appropriate elution conditions for your application.

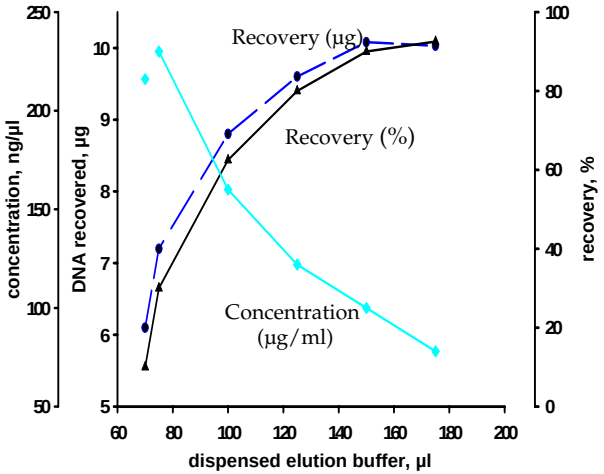

Figure legend: Recovery and concentration graph of plasmid DNA purified using the PureLink™ Quick96 Plasmid Kit. High DNA recovery is achieved with a volume of 120-125 µl of elution buffer.

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Recovery of Elution Volume

Recovery of elution volume varies based on the volume of PureLink™ Quick96 Elution Buffer used for elution.

The table below presents correlations between the volume of elution buffer used and the typical recoveries, when following kit protocol.

Note: The optimal volume of elution buffer for the PureLink™ Quick96 kits is 125 µl.

Elution buffer volume	Recovered elution buffer (all results are ±5 µl)
75 µl	30 µl
100 µl	55 µl
125 µl	80 µl
150 µl	105 µl
175 µl	130 µl

Processing Fewer than 96 Samples

You can use a portion of the PureLink™ Quick96 Plasmid Filter and Binding Plates to isolate plasmid DNA from fewer than 96 samples. **Each well in the plates can only be used once**.

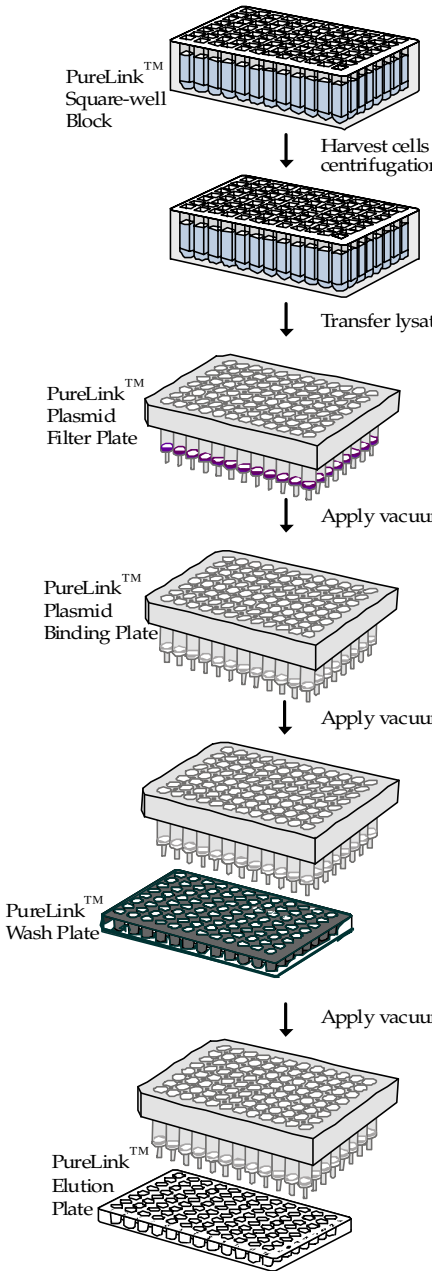
To process fewer than 96 samples:

1. Cover the entire surface of the filter plate **or** binding plate with Foil Tape (see page viii).
2. Just prior to filtering the lysate through the filter plate, or binding the DNA in the binding 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.

Important: 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.

Use a new plate with each experiment.

Experimental Overview



Grow cells overnight in LB medium with antibiotic.

Perform resuspension, lysis, and neutralization.

Filter the lysate using a PureLink™ Plasmid Filter Plate into a PureLink™ Plasmid Binding Plate.

Bind the DNA to the silica membrane of the Binding Plate.

Optional: Wash samples once with Wash Buffer I.

Required: Wash samples **twice** with Wash Buffer II.

Elute the plasmid DNA into the Purelink™ Elution Plate.

Equipment Compatibility

Introduction

The PureLink™ Quick96 Plasmid Kits are designed for use with vacuum manifolds. The purification steps can be completed manually or with automated liquid handling systems. The Quick96 Plasmid kits may also be used with certain centrifuges if higher concentrations of final DNA are required for downstream applications.

A list of equipment compatible with the PureLink™ Quick96 Plasmid Kits is provided below.

Vacuum Manifolds

The PureLink™ Quick96 Plasmid Kits can be used manually with any vacuum manifold that can accommodate PureLink™ Plates (see page 3). For manual applications, we recommend using the PureLink™ Vacuum Manifold to obtain optimal results with the Quick96 Plasmid Kits (see page vii).

Contact **Technical Support** (page 32), if you have questions concerning the use of your vacuum manifold.

Automated Liquid Handling Systems

The PureLink™ Quick96 Plasmid Kits are designed for use with automated liquid handling systems that allow the use of vacuum and a vacuum manifold. Some vacuum manifolds used on automated systems may require the PureLink™ Adapter Frame to ensure proper spacing and alignment of the PureLink™ Plates.

A list of compatible automated systems is provided below.

Note: Newer models of workstations are currently under evaluation. Contact **Technical Support** (page 32) for questions.

Automated System(s)	**Company**
Biomek® 2000/FX	Beckman-Coulter
BioRobot® 9600/3000/8000	Qiagen
Microlab® STAR	Hamilton
Miniprep™, Genesis™ RSP/RWS, Freedom Series	Tecan
MultiPROBE® II/II HT, JANUS™	Perkin Elmer
RoboSmart/RoboPrep/TheOnyx	Aviso
SciClone ALH	Zymark

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Centrifuges

Read this section before proceeding with centrifugation.

Certain centrifuges can be used to elute the final plasmid DNA if higher concentrations of purified final DNA are required for downstream applications.

Certain centrifuges can also be used to complete all purification steps instead of using vacuum.

The PureLink™ Quick96 Plasmid Kits use a plate stack with a height of **8.5 cm**. Most rotors are **not** able to accommodate an 8.5 cm high plate stack.

The following criteria must be met for successful purification using centrifugation:

- Centrifuge uses a rotor that accommodates an 8.5 cm plate stack.
- Centrifuge speed is 5,600 x g when combined with this rotor.
- You must use 2 ml square-well collection blocks that withstand 5,600 x g centrifugal forces without cracking.

Centrifuges and rotors that meet these requirements are available from Sigma and Beckman-Coulter.

Important: Failure to use a centrifuge that meets these requirements will cause the microtiter plates to break under the centrifugal force and cause cross-contamination in the samples.

Contact **Technical Support** (page 32) if you have questions concerning the use of a centrifuge with the PureLink™ Quick96 Plasmid Kits.

Before Starting

Introduction

Review the information in this section before starting the purification process.

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:* Water bath (37°C)

Preparing Resuspension Buffer with RNase A

Prepare the PureLink™ Quick96 Resuspension Buffer with the PureLink™ Quick96 RNase A as described below:

1. Add 1 ml Resuspension Buffer to each lyophilized RNase A vial and vortex until the RNase is dissolved.

   Note: The 4 x 96 kit contains only one vial of RNase A. The 24 x 96 kit contains five vials of RNase A.

2. Transfer entire contents from the vial(s) of now resuspended RNase A into the Resuspension Buffer bottle. Mix thoroughly. For the 24 x 96 kit, ensure **all five vials** of RNase A are transferred to the Resuspension Buffer.

3. Mark the label on the Resuspension Buffer to indicate that RNase A has been added.

4. **After mixing, store the Resuspension Buffer containing the RNase A at 4°C for up to six months. Equilibrate the mixed buffer to room temperature prior to using for purification.**

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.

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Preparing Wash Buffer II

Prepare the 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 x 96 preps

- Add 800 ml 96% ethanol to the 200 ml Wash Buffer II bottle.
- Mark the label on the bottle to indicate that ethanol has been added.

For 24 x 96 preps

1. Transfer 200 ml Wash Buffer II concentrate into the **reusable** dilution bottle (supplied with kit).
2. Add 800 ml 96% ethanol to the dilution bottle containing the 200 ml Wash Buffer II concentrate.

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

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.

Growing Cells and Preparing Lysates

Introduction

Instructions for growing bacterial cells and preparing lysates for purification are provided in this section.

Materials Needed

- Growth media (see **Recommended Media**, below)
- Appropriate antibiotic
- Centrifuge with rotor capable of spinning a 4.5 cm square-well block at 1,000 x g
- Shaking incubator at 37°C (200-250 rpms is optimal)
- *Optional*: Culture flask

Components supplied with the kit:

- PureLink™ Quick96 Square-well Block
- PureLink™ Quick96 Resuspension Buffer containing RNase A (see page 10)
- PureLink™ Quick96 Lysis Buffer
- PureLink™ Quick96 Neutralization Buffer
- Gas-permeable Foil

Recommended Media

The PureLink™ Quick96 Plasmid kits are designed to isolate plasmid DNA from 1.5 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.

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Recommended Cell Volume

The recommended cell volume for use with the PureLink™ Quick96 Plasmid Kits is 1.2-1.5 ml of cells per well.

Use the recommended cell volume for plasmid isolation.

Using cell volumes >1.5 ml may result in inefficient cell lysis or cross-contamination of samples.

Plasmid Copy Number

The PureLink™ Quick96 Plasmid 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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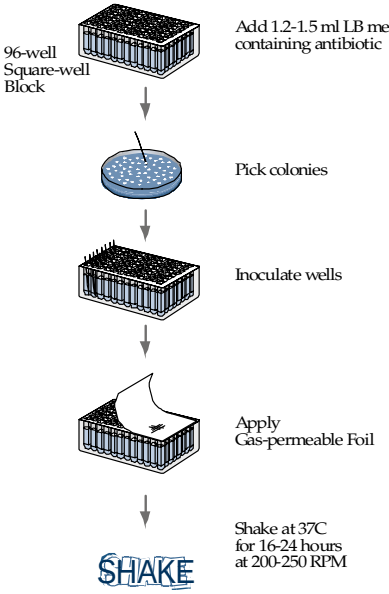
Preparing 96-Well Cultures

Use the supplied PureLink™ Quick96 Square-well 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 1.2-1.5 ml of the LB medium containing the antibiotic into each well of the square block.

   Note: To avoid cross-contamination due to spilling during incubation, do not exceed a total culture volume of 1.5 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 square-well block.
4. Repeat for all 96 wells of the square-well block. Remove toothpicks and discard into a biowaste container.
5. Cover the square-well block securely with the supplied gas-permeable foil.
6. Incubate the block containing the cultures in a 37°C 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 16).

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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 rpms 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 rpms.
5. Proceed to **Harvesting Cells and Preparing Lysates**, page 16.

Important

- For harvesting the bacterial cells (next page), centrifuge the cells at 1,000 x g. Centrifugation at higher forces may produce tight pellets which are more difficult to resuspend.

  Note: If centrifugation at higher g-forces is used, a shaker integrated on the automated liquid handling station may be necessary for complete resuspension of the cell pellets, after addition of the Resuspension Buffer with RNase A.
- 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.

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Harvesting Cells and Preparing Lysates

To prepare the bacterial lysate:

1. Harvest the cells as follows:

   Square-well Block: Remove the sealed square-well block from the shaking incubator and place it in the centrifuge rotor. Centrifuge at 1,000 x g for 10 minutes.

   Culture Flask: Remove the flask from the shaking incubator and transfer 1.2-1.5 ml of culture cells into each well of the PureLink™ Quick96 Square-well Block (supplied with kit). Seal the square-well block with Foil Tape (page viii) and place the square-well block in the centrifuge rotor. Centrifuge at 1,000 x g for 10 minutes.

2. Remove the square-well block from the centrifuge. Discard the seal and remove the media from the wells.
3. Add 250 µl Resuspension Buffer with RNase A (page 10) into each well of the square-well block. Shake the square-well block gently until cell suspension is homogeneous. No cell clumps should be visible.
4. Add 250 µl Lysis Buffer to each well of the square-well block. Shake the block **gently** to mix. **Do not** mix by pipetting up and down.
5. Incubate the bacterial lysate at room temperature for 2-5 minutes. **Do not exceed 5 minutes.**
6. Add 350 µl Neutralization Buffer into each well of the square-well block. Mix by **gently** pipetting up and down.
7. Proceed to **Filtering Lysate and Binding DNA** (page 20).

Purification Process Using Vacuum

Introduction

Instructions are provided below to manually isolate plasmid DNA from overnight bacterial cultures, using vacuum. The compatibility of the PureLink™ Plates with various instruments is described on page 8.

Perform all purification steps at room temperature.

Note: A support protocol for completing all purification steps using centrifugation is provided in the Appendix (page 25).

Note

If higher concentrations of final DNA are required for downstream applications, an optional protocol to elute the plasmid DNA by centrifugation is provided on page 23.

Use of a centrifuge for eluting DNA reduces the volume of elution buffer used to 50-75 µl.

To elute the final DNA using centrifugation, complete all purification steps with vacuum through the second wash step, then proceed to **Eluting DNA With Centrifugation.** (page 23).

Important: Ensure the centrifuge meets all requirements before beginning the purification process (see page 9).

Materials Needed

- Vacuum manifold or automated liquid handling system
- Suitable vacuum source (house vacuum, vacuum pump, or water aspirator) producing a vacuum pressure of -200 to -400 millibars (mbars)
- *Optional*: Centrifuge that meets specific requirements (see page 9)
- *Optional*: Sterile, distilled water (pH 8.0-8.5)

Components supplied with the kit:

- PureLink™ Quick96 Plasmid Filter Plate
- PureLink™ Quick96 Plasmid Binding Plate
- PureLink™ Quick96 Wash Plate
- PureLink™ Quick96 Elution Plate
- *Optional*: PureLink™ Quick96 Wash Buffer I
- PureLink™ Quick96 Wash buffer II prepared with ethanol (see page 11)
- *Optional:* Paper sheets
- PureLink™ Quick96 Elution Buffer

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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 1-3 minute incubation with elution buffer.
- Increase elution buffer volume up to 150 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 8.0-8.5.

Assembly of the Vacuum Manifold

General assembly instructions for the PureLink™ Vacuum Manifold are provided below. To operate the vacuum manifold, refer to the manifold's instruction manual.

1. Place the manifold on a secure lab bench or work area.
2. Place a waste tray inside the manifold base and insert the appropriate spacers (notch side up) completely inside the grooves of the manifold base.
3. Place the manifold lid on top of the manifold base.
4. Connect the manifold to a suitable vacuum source (*e.g.*, house vacuum, vacuum pump, water aspirator) and calibrate the vacuum pressure (next page).

Continued on next page

Calibrating Vacuum for Use with 96-Well Plates

For optimal results using vacuum, calibrate the vacuum pressure prior to using the PureLink™ Quick96 Plasmid Kits.

A vacuum regulator is required for this step (see page viii).

Note: For 96-well plates, a vacuum pressure of -200 to -400 mbars (-6 to -12 inches Hg or -150 to -300 mm Hg) is recommended.

To calibrate the vacuum pressure:

1. Assemble the vacuum manifold according to the manufacturer's specifications.
2. Place an unused 96-well microtiter plate in the manifold lid. Seal the plate with Foil Tape (page viii).
3. Connect the vacuum regulator between the manifold base and the vacuum source. Close the manifold valve.
4. Turn on the vacuum source and adjust the vacuum regulator to achieve the desired pressure reduction. Open the manifold valve to reduce the pressure in manifold.
5. Close the manifold valve to release vacuum pressure in the manifold and begin plasmid DNA purification.

Continued on next page

Filtering Lysate and Binding DNA

Instructions for filtering the crude lysate and binding the DNA using vacuum are provided below.

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™ Quick96 Plasmid Binding Plate inside the vacuum manifold base and place the manifold lid on top.

3. Insert a PureLink™ Quick96 Plasmid Filter Plate into the manifold lid. Align the filter plate with the binding plate.

   Note: If processing fewer than 96 samples, cover unused wells of the filter plate with Foil Tape (see page 6).

4. Transfer the lysate (see page 16) to the filter plate using a multichannel pipettor or robotic loading device.

5. Apply vacuum for 1-5 minutes until all lysate passes through the filter plate. Use a vacuum pressure of -200 to -400 mbars or reduce the vacuum pressure until a filtration rate of 1-2 drops per second is attained.

6. Release vacuum pressure and **discard** the filter plate.

7. Remove the manifold lid and take out the binding plate from the manifold base. Replace the lid onto the base and insert the binding plate into the manifold lid.

   Note: If processing fewer than 96 samples, cover unused wells of the binding plate with Foil Tape (see page 6).

8. Apply vacuum for 1 minute to bind the DNA from the lysate to the silica membrane of the binding plate. Use a vacuum pressure of -200 to – 400 mbars or reduce the vacuum pressure until a flow rate of 1-2 drops per second is attained.

9. Ensure all cleared lysate passes through the membrane of the binding plate and release vacuum.

10. Proceed to **Washing DNA** (next page).

Continued on next page

Note

The PureLink™ Quick96 Plasmid Kits contain two wash buffers, PureLink™ Quick96 Wash Buffer I and PureLink™ Quick96 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 binding plate and the manifold lid. Place a PureLink™ Quick96 Wash Plate (page 3) into the manifold base. Replace the manifold lid and insert the binding plate into the lid.
2. **Optional:** Add 600 µl Wash Buffer I into each well of the binding plate.
3. Apply vacuum for 1 minute to pass all buffer through the binding plate. Use a vacuum pressure of -200 to -400 mbars or reduce the pressure until a flow rate of 1-2 drops per second is attained.
4. **Required:** Add 900 µl of prepared Wash Buffer II with ethanol (page 11) into each well of the binding plate.
5. Apply vacuum for 1 minute to pass all buffer through the binding plate. Use a vacuum pressure of -200 to -400 mbars or reduce the pressure until a flow rate of 1-2 drops per second is attained.
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.

   Proceed to **Eluting DNA with Centrifugation** (page 23) if you are using a centrifuge to elute the final DNA.
8. Remove the manifold lid and the binding plate. Remove and discard the wash plate from the base and replace the manifold lid. Insert the binding plate into the lid.
9. Apply vacuum for 10 minutes at a vacuum pressure of -400 mbars to remove any residual ethanol from the binding plate. The ethanol in the Wash Buffer II may inhibit downstream enzymatic reactions, and should be completely removed. Release vacuum.
10. *Optional*: Remove the binding plate from the manifold lid and blot the bottom nozzles of the plate on paper sheets (supplied with kit) to remove any residual ethanol.
11. Proceed to **Eluting DNA Using Vacuum** (next page).

Continued on next page

Note

The PureLink™ Quick96 Elution Buffer contains 5 mM Tris-HCl, pH 8.5). Using an elution buffer with a Tris-HCl concentration higher than 10 mM can interfere with some downstream reactions.

If the Tris-HCl in the elution buffer interferes with your downstream applications, use sterile, distilled water (pH 8.0-8.5) for elution.

Eluting DNA Using Vacuum

1. After washing the samples and drying the binding plate (page 21), remove the manifold lid and the binding plate from the manifold base.
2. Remove and discard the wash plate from the manifold base.
3. Place a PureLink™ Quick96 Elution Plate into the manifold base and replace the manifold lid. Insert the binding plate into the lid and align the binding plate with the elution plate.
4. Add 125 µl Elution Buffer (5 mM Tris-HCl, pH 8.5) or sterile, distilled water (pH 8.0-8.5) into the center of each well of the binding plate.

   Note: You can use an elution buffer volume of 75-150 µl (see page 5 for elution parameters).
5. Incubate the binding plate with elution buffer at room temperature for 1-3 minutes.
6. Apply vacuum for 1 minute to elute the DNA from the binding plate into the elution plate. Use a vacuum pressure of -200 to -400 mbars or reduce the vacuum pressure until a flow rate of 1-2 drops per second is attained.
7. Release vacuum and remove the elution plate from the manifold base.
8. Seal the elution plate with adhesive cover foil (supplied with kit). Store the eluted DNA at -20°C (long-term), or 4°C (short-term), or proceed to downstream application.

Continued on next page

Important

Review the centrifuge and square-well block requirements (page 9) **before** beginning the elution steps with centrifugation.

Additional 1-2 ml square-well blocks are required for eluting DNA with centrifugation and **must be purchased separately**.

Eluting DNA with Centrifugation

1. Stop the purification process using vacuum after you complete the final wash step (Step 6, page 21). Do not continue on to dry the binding plate using vacuum.
2. Remove the PureLink™ Quick96 Plasmid Binding Plate from the vacuum manifold lid and gently tap the nozzles on the bottom of the plate on paper sheets (supplied with kit) to remove any residual ethanol, as the ethanol can affect some downstream applications.
3. Stack the binding plate on top of a **new** 1-2 ml 96-well square-well block (page viii). **Do not** use the square-well block used to grow overnight bacterial cultures.

   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.
4. Place the plate stack inside a centrifuge with the appropriate rotor. Centrifuge for 10 minutes at 5,800 x g to dry the silica membrane of the binding plate and remove any residual ethanol.
5. Remove the plate stack from the centrifuge. Empty the flow-through into an appropriate waste container and **discard** the square-well block.
6. Stack the binding plate on a **new** 1-2 ml square-well block.
7. Add 50-75 µl Elution Buffer directly onto the silica membrane of each well in the binding plate.
8. Incubate samples in elution buffer at room temperature for 1-3 minutes.
9. Place the plate stack into the centrifuge and centrifuge for 2 minutes at 5,800 x g to elute the DNA from the binding plate into the square-well block underneath.
10. Remove the plate stack from the centrifuge and seal the square-well block containing the plasmid DNA with adhesive cover foil (supplied with kit).
11. 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™ Quick96 Plasmid Kits is easily quantitated using UV absorbance at 260 nm or using Quant-iT™ DNA Assay Kits (see page vii).

UV Absorbance

1. Measure the A_{260} of the solution using a spectrophotometer blanked against 5 mM Tris-HCL, pH 8.5.

   Note: you 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} x 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™ Quick96 Plasmid Kits has 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.

Appendix

Support Protocol for Purification Process using Centrifugation

Introduction

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

Before beginning: The PureLink™ Quick96 Plasmid Kits use a plate stack with a height of **8.5 cm**. Ensure **all** equipment meets the specific requirements provided on page 9.

Perform all purification steps at room temperature.

Note: The PureLink™ Quick96 Plasmid Kits are designed for rapid, high-throughput purification of Plasmid DNA using vacuum. Use of a centrifuge to complete all purification steps significantly increases the processing time.

Materials Needed

- A room temperature centrifuge with a rotor capable of holding plate stacks **8.5 cm** high and having a speed of 5,800 x g (see page 9)
- **2 ml 96-well, Square-well Blocks** (see page viii), capable of withstanding centrifugal forces of 5,800 x g (see page 9)
  Note: Additional **2 ml** 96-well square-well blocks are required for the centrifugation steps and **must be purchased separately**
- *Optional:* Sterile, distilled water (pH 8.0-8.5)

Contents supplied with the kit:

- PureLink™ Quick96 Plasmid Filter Plate
- PureLink™ Quick96 Plasmid Binding Plate
- PureLink™ Quick96 Wash Plate
- PureLink™ Quick96 Elution Plate
- *Optional*: PureLink™ Quick96 Wash Buffer I
- PureLink™ Quick96 Wash buffer II prepared with ethanol (see page 11)
- PureLink™ Quick96 Elution Buffer

Continued on next page

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 1-3 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 8.0-8.5.

Use a **2 ml** 96-well, square-well collection block for the plate stack (page viii). The square-well block must be capable of withstanding centrifugal forces of 5, 800 x g.

Using a 2 ml 96-well square-well block allows the nozzles of the PureLink™ Quick96 Plasmid Filter and Binding Plates to be inserted into the wells of the square-well block without contacting the buffer flow-through in the wells. This prevents cross-contamination of the samples and allows for proper purification of the plasmid DNA.

Using a 1 ml square-well block is allowed **only** for the elution step, as the volume of elution buffer used is low.

Continued on next page

Support Protocol for Plasmid Purification using Centrifugation, Continued

Filtering Lysates and Binding DNA

Instructions for filtering the crude lysate and binding the DNA using centrifugation are provided below.

1. Harvest the overnight bacterial cell culture, then resuspend and lyse the cells according to the protocol on page 16.
2. Stack a PureLink™ Quick96 Plasmid Filter Plate on top of a **new** 2 ml 96-well, square-well block (see page viii). **Do not** use the square-well block used to grow the overnight bacterial cell cultures.
3. Transfer the lysates from Step 1 (above) into the filter plate with a multichannel pipettor.
4. Place the plate stack into the centrifuge and centrifuge for 4 minutes at 5,800 x g to filter the lysate into the wells of the square-well block.
5. Remove the plate stack from the centrifuge and **discard** the filter plate.
6. Stack a PureLink™ Quick96 Plasmid Binding Plate on top of a **new** 2 ml 96-well square-well block.
7. Transfer the filtered lysates from the square-well block in Step 4 into the wells of the binding plate using a multichannel pipettor.
8. Place the plate stack into the centrifuge and centrifuge the stack for 4 minutes at 5,800 x g to bind the DNA to the silica membrane of the binding plate.
9. Remove the plate stack from the centrifuge. Empty the flow-through from the square-well block into an appropriate waste container.
10. Proceed to **Washing DNA** (next page).

Continued on next page

Support Protocol for Plasmid Purification using Centrifugation, Continued

Note

The PureLink™ Quick96 Plasmid Kits contain two wash buffers, PureLink™ Quick96 Wash Buffer I and PureLink™ Quick96 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 binding plate on top of the square-well-block from the previous step (Step 9, page 27).
2. *Optional:* Add 600 µl Wash Buffer I to each well of the binding plate.
3. Place the plate stack into the centrifuge and centrifuge for 4 minutes at 5,800 x g.
4. Remove the plate stack and empty the flow-through from the square-well block into an appropriate waste container. Restack the binding plate on top of the square-well block.
5. *Required*: Add 900 µl of prepared Wash Buffer II (see page 11) into each well of the binding plate.
6. Place the plate stack into the centrifuge and centrifuge for 4 minutes at 5,800 x 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 square-well block into an appropriate waste container.
9. Restack the binding plate on top of the square-well block. Place the plate stack into the centrifuge to dry the silica membrane and remove any residual ethanol, as the ethanol can inhibit some downstream applications.
10. Centrifuge the plate stack for 10-15 minutes at 5,800 x g.
11. Remove the plate stack from the centrifuge. Empty the flow-through into an appropriate waste container and **discard** the square-well block.
12. *Optional:* Gently tap the bottom nozzles of the binding plate on paper sheets (included with kit) to remove any remaining ethanol traces from the nozzles.
13. Proceed to **Eluting DNA** (next page).

Continued on next page

Support Protocol for Plasmid Purification using Centrifugation, Continued

Eluting DNA

1. Stack the now dried binding plate (page 28) on top of a **new** 1-2 ml 96-well square-well block.
2. Add 50-150 µl Elution Buffer directly onto the silica membrane of each well in the binding plate.

   Note: Use a low volume of elution buffer for higher DNA concentrations. Use a higher volume of DNA for higher DNA yield (see **Elution Parameters** on page 5 for details).
3. Incubate samples in elution buffer for 1-3 minutes.
4. Place the plate stack into the centrifuge and centrifuge for 2 minutes at 5,800 x g to elute the DNA from the binding plate into the square-well block underneath.
5. Remove the plate stack from the centrifuge. *The eluted DNA is in the square-well block.*
6. Seal the square-well block containing the plasmid DNA with adhesive cover foil (supplied with kit). Store eluted DNA at -20°C (long-term) or 4°C (short-term), or proceed to the downstream application.

Troubleshooting

Introduction

The table below describes solutions to possible problems you may experience with the PureLink™ Quick96 Plasmid Kits. For additional assistance, contact **Technical Support** (see page 32).

Problem	Cause	Solution
Incomplete lysis of bacterial cells	Too many cells used	• Reduce bacterial culture volume to 1.0-1.5 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 13).
	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 12).

Continued on next page

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 11). • Ensure the lid on the buffer is tightly closed.
	Poor elution conditions	• Use PureLink™ Quick96 Elution Buffer, (5 mM Tris-HCl, pH 8.5). • If using nuclease-free water for elution, ensure pH values are within pH 8.0-8.5. **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 12 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 filter 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).

Technical Support

Web Resources

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**).

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MSDS

MSDSs (Material Safety Data Sheets) are available on our website at **www.invitrogen.com/msds.**

Certificate of Analysis

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

Purchaser Notification

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