invitrogen™

PureLink™ HQ 96 Plasmid DNA Purification Kit

For high-throughput isolation of high-quality plasmid DNA

Catalog no. K2100-96

Version C
5 May 2006
25-0676

User Manual

Table of Contents

Experienced Users Procedure

Introduction

This quick reference sheet is included for experienced users of the PureLink™ HQ 96 Plasmid DNA Purification Kit. If you are a first time user, follow the protocol from this manual.

Step	Action
Grow Cells	Grow cells at 37ºC overnight in a culture flask or a 96-well growth block using LB medium with appropriate antibiotic.
Prepare Lysates	1. Harvest cells by centrifugation at 1,500 × g at 4ºC for 5-10 minutes. Remove growth media. 2. Resuspend cell pellets in 200 µl Resuspension Buffer with RNase A. Add 200 µl Lysis Buffer to each well. Mix well. 3. Incubate at room temperature for 3 minutes. 4. Add 300 µl of Neutralization/Binding Buffer. Mix well.
Purification Protocol Using Vacuum	1. Set up the vacuum manifold. Use a vacuum pressure of –600 to –700 millibars (mb) (–450 to –525 mm Hg). 2. Place a PureLink™ HQ 96 Binding Plate inside the vacuum manifold base. Place a PureLink™ HQ 96 Clarification Plate on top of the manifold. 3. Transfer ~700 µl lysate, above, to the Clarification Plate. 4. Apply vacuum for 1 minute. Release the vacuum. Discard Clarification Plate. 5. Transfer the Binding Plate to the top of the vacuum manifold and place waste tray at the base of the manifold. 6. Apply vacuum for 1–2 minutes until all samples have passed through the Binding Plate. Release the vacuum. 7. Add 600 µl Wash Buffer with ethanol to the Binding Plate. 8. Apply vacuum for 2 minutes. Release the vacuum. **Repeat** Wash Step one more time. 9. Apply vacuum for 10 minutes. Release the vacuum. Tap the plate on a stack of paper towels to blot the plate dry. 10. Place the Receiver Plate in the vacuum manifold (in place of the waste collection tray) and place the Binding Plate on the manifold. 11. Add 100 µl Elution Buffer to the Binding Plate. 12. Incubate the plate at room temperature for 1 minute. 13. Apply vacuum for 2-5 minutes to elute the plasmid DNA into the Receiver Plate. Release the vacuum. 14. Store plasmid DNA at -20ºC.

Continued on next page

Experienced Users Procedure, Continued

Step	Action
Purification Protocol Using Centrifugation	Perform all centrifugation steps at room temperature using a centrifuge with a swinging bucket rotor with plate carriers that have a plate height clearance of ~7.0 cm. 1. Place a PureLink™ HQ 96 Clarification Plate on a 1.1 ml 96 deep-well collection plate. 2. Transfer ~700 µl lysate (prepared as described on the previous page) to the Clarification Plate. 3. Centrifuge the stacked plates at 1,800 × g for 1 minute. 4. Transfer flow through from the Clarification Plate onto the PureLink™ HQ 96 Binding Plate. Discard the Clarification Plate. 5. Place the PureLink™ HQ 96 Binding Plate from Step 4 on top of the collection plate. 6. Centrifuge the stacked plates at 1,800 × g for 3 minutes. 7. Add 600 µl Wash Buffer with ethanol to the Binding Plate. 8. Centrifuge the stacked plates at 1,800 × g for 3 minutes. Discard the flow through**.** 9. **Repeat** the Wash Step one more time. Discard the flow through. 10. Place the Binding Plate on top of a dry collection plate and centrifuge the stacked plates at 1,800 × g for 5 minutes. 11. Place the PureLink™ HQ 96 Binding Plate on top of a new Receiver Plate. 12. Add 100 µl Elution Buffer to the Binding Plate. 13. Incubate the plate at room temperature for 1 minute. 14. Centrifuge the stacked plates at 1,800 × g for 3 minutes to elute the plasmid DNA. 15. Store plasmid DNA at -20ºC.

Kit Contents and Storage

Shipping and Storage

All components of the PureLink™ HQ 96 Plasmid DNA Purification Kit are shipped at room temperature. Upon receipt, store all components at room temperature.

After addition of RNase, store the Resuspension Buffer with RNase at 4°C.

Contents

The components included in the PureLink™ HQ 96 Plasmid DNA Purification Kit are listed below.

Sufficient reagents are provided in the kit to perform 384 (4 × 96) isolations.

Component	Amount
Resuspension Buffer (R1)	120 ml
Lysis Buffer (L1)	120 ml
Neutralization/Binding Buffer (B1)	2 × 85 ml
Wash Buffer (W1)	120 ml
Elution Buffer; 10 mM Tris-HCl, pH 8.5 (E1)	70 ml
RNase A	12 mg
PureLink™ HQ 96 Clarification Plate	4
PureLink™ HQ 96 Binding Plate	4
Receiver Plate	4
Adhesive Foil for plates	20

Product Qualification

The PureLink™ HQ 96 Plasmid DNA Purification Kit is functionally qualified by isolating a high-copy number plasmid DNA from 1.0×10^9 *E. coli* cells as described in this manual. The kit must produce the following results:

- $A_{260}/A_{280} \geq 1.80$
- Genomic DNA or RNA contamination not detected when 10% of eluted volume is analyzed by gel electrophoresis
- Low endotoxin level as measured against a standard curve using a kinetic turbidometric method (for more details, refer to the certificate of analysis)

In addition, each kit component is sterile and lot qualified for optimal performance.

Accessory Products

Additional Products

The following products are also available from Invitrogen. For more details on these products, visit our Web site at www.invitrogen.com or contact Technical Support (page 29).

Product	Quantity	Catalog no.
PureLink™ HQ Mini Plasmid DNA Purification Kit	100 reactions	K2100-01
PureLink™ HiPure Plasmid Filter Midiprep Kit	25 preps 50 preps	K2100-14 K2100-15
PureLink™ HiPure Plasmid Filter Maxiprep Kit	25 preps 50 preps	K2100-16 K2100-17
Growth Blocks	Pack of 50	12256-020
Air porous Tape	Pack of 50	12262-010
Luria Broth Base, powder (Miller's Luria Broth Base)	500 g	12795-027
Ampicillin	200 mg	11593-019
Carbenicillin, Disodium salt	5 g	10177-012
Zeocin™	1 g	R250-01
SequenceRx Enhancer System	25 reactions	12237-012
Lipofectamine™ 2000 Transfection Reagent	0.75 ml	11668-027
Platinum® PCR SuperMix High Fidelity	100 reactions	12532-016
Platinum® Blue PCR SuperMix	100 reactions	12580-015
AccuPrime™ *Pfx* SuperMix	200 reactions	12344-040
PCR SuperMix	100 reactions	10572-014
E-Gel® *96* 1% Gels	8 gels	G7008-01
E-Gel® *96* 2% Gels	8 gels	G7008-02
UltraPure™ DNase/RNase-free Distilled Water	500 ml	10977-015
Quant-iT™ DNA Assay Kit, High Sensitivity	1000 assays	Q33140
Quant-iT™ DNA Assay Kit, Broad-Range	1000 assays	Q33130
PureLink™ 96 Receiver Plate	50	12193-025

Introduction

Overview

Introduction

The PureLink™ HQ 96 Plasmid DNA Purification Kit is designed for high-throughput isolation of high-quality plasmid DNA from bacterial cells.

The high quality of isolated plasmid DNA is demonstrated by its low genomic DNA contamination, high supercoiled to nicked forms ratio, and reliable performance in demanding downstream applications such as mammalian cell transfection (see next page).

The PureLink™ HQ 96 Plasmid DNA Purification Kit can be used with a vacuum manifold or a centrifuge and is compatible with automated liquid handling workstations (page 14).

System Overview

Bacterial cells are harvested and resuspended in Resuspension Buffer with RNase. The cells are lysed using an alkaline/SDS cell lysis procedure. The lysate is then neutralized and conditions are adjusted for subsequent binding. After lysate clarification using the PureLink™ HQ 96 Clarification Plate, the lysate is processed through the PureLink™ HQ 96 Binding Plate. The DNA binds to the silica-based membrane in the plate and impurities are removed by washing with Wash Buffer. The DNA is then eluted in low salt Elution Buffer or water.

Advantages

The PureLink™ HQ 96 Plasmid DNA Purification Kit offers the following advantages:

- Ability to isolate up to 10 µg plasmid DNA per well
- Designed to isolate high-quality plasmid DNA in 30-45 minutes
- Minimal genomic DNA contamination in the purified plasmid DNA
- Compatible with automated liquid handling workstations
- Reliable performance of the purified plasmid DNA in downstream applications such as mammalian transfection

Continued on next page

Downstream Applications

The plasmid DNA isolated using the PureLink™ HQ 96 Plasmid DNA Purification Kit is suitable for a variety of downstream applications such as:

- Restriction enzyme digestion
- PCR
- Sequencing
- Bacterial transformation
- Mammalian transfection

For more details on applications, see page 26.

Product Specifications

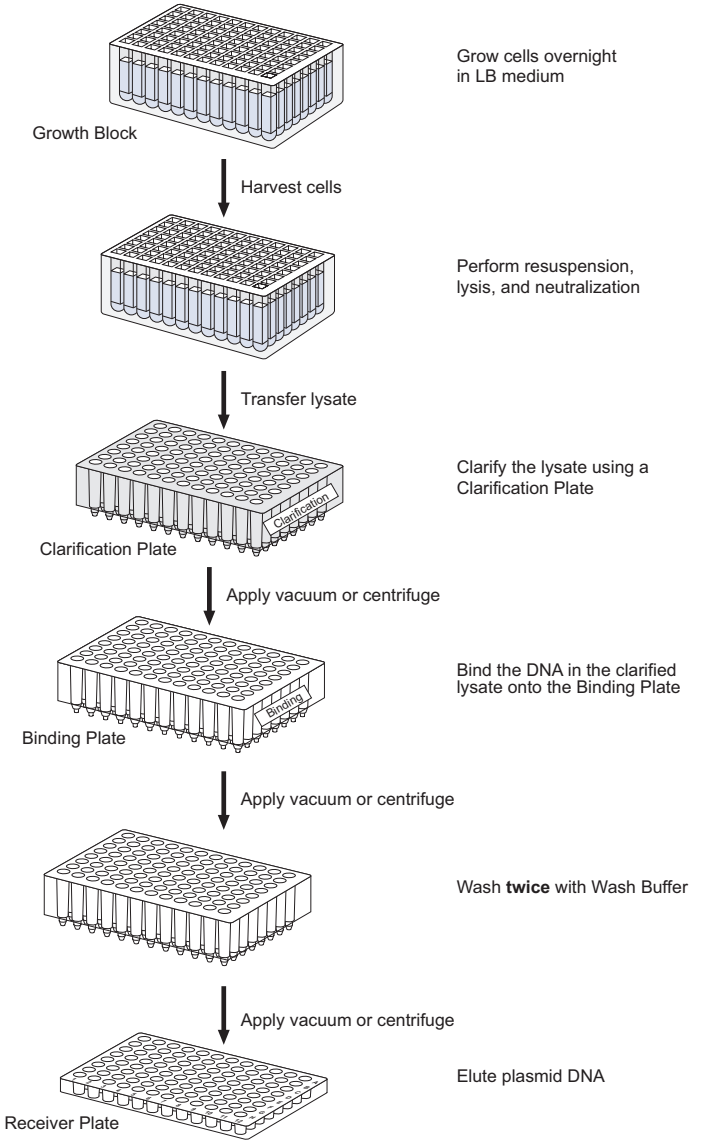
PureLink™ HQ 96 Binding Plate	Dimensions:	Standard SBS (Society for Biomolecules Screening) footprint
	Description: long	Polypropylene 96-well plate with drip director
	Volume:	1.3 ml
	Binding Capacity:	10 µg dsDNA/well
PureLink™ HQ 96 Clarification Plate	Dimensions:	Standard SBS (Society for Biomolecules Screening) footprint
	Description:	Polypropylene 96-well plate with large orifice long drip director
	Volume:	1.3 ml
Receiver Plate	Dimensions:	Standard SBS (Society for Biomolecules Screening) footprint
	Description:	Polypropylene 96-well round bottom plate with a polystyrene lid
	Volume:	100 µl
System Specifications	Starting Material:	1-3 ml (1-1.5 × 10^9 cells) lysate/well
	Elution Volume:	100 µl
	Recovery:	>90%
	Instrument Compatibility	Vacuum manifold or centrifuge (page 14)

Experimental Overview

Introduction

The flow chart describes the steps for isolating plasmid DNA.

Note: The PureLink™ HQ 96 Clarification Plate is shaded in the figure below for illustration only. The actual Clarification Plate is clear.



Methods

Before Starting

Materials Needed

- 96-100% ethanol
- Water bath (37ºC), optional

Preparing Resuspension Buffer with RNase

Prepare the Resuspension Buffer with the RNase A as described below:

1. Resuspend the lyophilized RNase A (12 mg) included in the kit in 200 µl Resuspension Buffer.
2. Add the resuspended RNase mixture to the remaining Resuspension Buffer for a final concentration of 0.1 mg/ml RNase A.
3. **After mixing, store the Resuspension Buffer with RNase A at 4°C. Stable for up to 6 months.**

Preparing Wash Buffer

To 120 ml Wash Buffer included in the kit, add 480 ml 96-100% ethanol. Mix well and store at room temperature.

Note

Before each use, check the Neutralization/Binding Buffer and Lysis Buffer supplied with the kit for a white salt precipitate. If precipitate is present, place each buffer in a 37°C water bath for 5 minutes or until the salts redissolve and the solution clears.

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

Continued on next page

Processing Fewer than 96 Samples

You can use a portion of the PureLink™ HQ 96 Binding Plate, if you wish to isolate plasmid DNA from less than 96 samples. **Each well can only be used once.**

1. Cover the entire surface of the Binding Plate with adhesive foil.
2. Just prior to use, use a sharp blade to score the foil around the wells to be used and peel away the foil to expose the clean wells.

   Important: Keep all unused wells sealed with adhesive foil during purification to obtain uniform vacuum.

You will need a new Receiver plate for each experiment.

Instrument Compatibility

The PureLink™ Plates are compatible with the following instruments:

- **Vacuum Manifold:** The manifold must accommodate the PureLink™ Plates and be capable of collecting the filtrate (e.g. UniVac 3 Vacuum Manifold System from Whatman)
- **Centrifuge:** The centrifuge must be capable of centrifuging 96-well plates 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. The PureLink™ HQ 96 Plasmid DNA Purification Kit has been tested and qualified for use on the Biomek® FX Automation Workstation and the Tecan Freedom EVO™ Workstation.

  For the Biomek® FX, we recommend using the Small Vacuum Adapter Collar (Whatman cat. no. 7705-0120) and Medium Vacuum Adapter Collar (Whatman cat. no. 7705-0121) with PureLink™ Plates.

For more information about compatibility with instruments and robotic workstations, contact Invitrogen Technical Support (page 29).

Preparing Lysates

Introduction

Instructions for growing cells and preparing lysates are included in this section.

Materials Needed

- Resuspension Buffer with RNase (page 13)
- Lysis Buffer (supplied in the kit)
- Neutralization/Binding Buffer (supplied with the kit)
- Growth Block (cat. no 12256-020) or culture flask
- Growth media (see **Recommended Media**, below)
- Appropriate antibiotic
- Air porous tape (cat. no.12262-010)
- Shaking incubator at 37ºC

The buffers included in the PureLink™ HQ 96 Plasmid DNA Purification Kit contain guanidine hydrochloride and sodium hydroxide. Always wear a laboratory coat, disposable gloves, and eye protection when handling buffers.

Do not add bleach or acidic solutions directly to solutions containing guanidine hydrochloride or sample preparation waste as it forms reactive compounds and toxic gases when mixed with bleach or acids.

Plasmid DNA yield depends on a variety of factors:

- Plasmid copy number per cell
- Plasmid size
- Size of the insert
- Effect of the insert on plasmid propagation
- Bacterial culture growth

Follow the recommendations below to improve the plasmid DNA yield:

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

Continued on next page

Recommended Media

The PureLink™ HQ 96 Plasmid DNA Purification Kit is designed to isolate plasmid DNA from 1-3 ml *E. coli* cells grown overnight in **LB medium** in a 96-well growth block.

Note

If you are using rich medium such as Terrific Broth, you may need to adjust the volume of cells used for isolation to avoid clogging the PureLink™ HQ 96 Clarification Plate (see below).

Recommended Cell Number and Volume

The recommended cell number for use with the PureLink™ HQ 96 Plasmid DNA Purification Kit is **1-1.5 × 10^9 cells/well** and the recommended cell volume is **1-3 ml**.

Using cell numbers >1.5 × 10^9 cells/well will result in well-to-well variability in plasmid recovery.

Using cell volumes >3 ml or cell numbers >3 × 10^9 cells/well may result in inefficient lysis and incomplete lysate clarification due to clogging of the PureLink™ HQ 96 Clarification Plate.

Plasmid Copy Number

The PureLink™ HQ 96 Plasmid DNA Purification Kit is designed to isolate plasmid DNA from **high-copy** number plasmids.

If you are using a low-copy number plasmid, avoid using rich medium such as Terrific Broth for growing cells. We recommend using LB medium for growth and use the recommended cell number for plasmid isolation.

Preparing Growth Culture in 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 37ºC overnight on a shaker.
3. Transfer the overnight culture to a 250-ml culture flask containing ~150 ml LB medium with appropriate antibiotic.
4. Grow cells at 37ºC overnight on a shaker.
5. Proceed to **Harvesting Cells and Preparing Lysates**, page 18.

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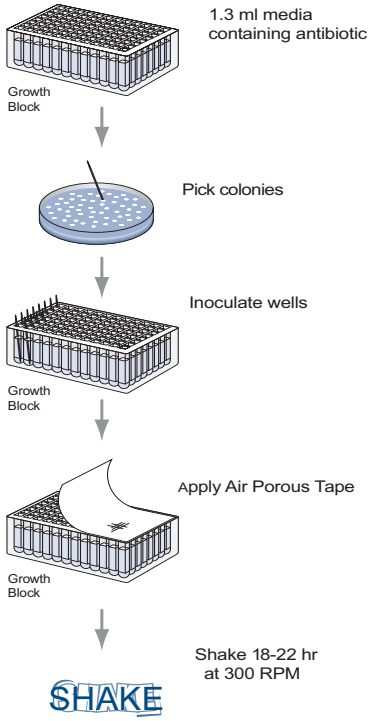
Preparing 96-Well Cultures

Follow the steps below to prepare your 96-well growth block.



1. Add an appropriate concentration of antibiotic to LB media.
2. Place 1.3 ml of antibiotic-containing LB media into each well of a growth block.
3. Pick a well-isolated colony (colonies should be no more than 2 weeks old) with a sterile toothpick. Drop the toothpick into a growth block well.
4. Repeat for all 96 wells of the growth block. Remove toothpicks and discard into a biowaste container.
5. Cover growth block with air porous tape. Seal cover securely.
6. Incubate in a 37°C shaker at 300 rpm for 18–22 hours.
7. Proceed to **Harvesting Cells and Preparing Lysates**, next page.

Continued on next page

Important

To prevent shearing of chromosomal DNA and contamination of plasmid DNA with chromosomal DNA, perform all mixing steps **gently** during lysis and neutralization.

We recommend covering the growth block or 96-well plate with the adhesive foil included in the kit and **mix gently** by inverting the block or plate.

If you are performing lysis on an automated liquid handling workstation, **mix gently** using a shaker or by gently pipetting up and down. Avoid vortexing the lysate.

Harvesting Cells and Preparing Lysates

To prepare the bacterial cell lysate:

1. Harvest cells as follows:

   Growth Block: Centrifuge the growth block at 1,500 × g for 5-10 minutes at 4ºC.

   Culture Flask: Transfer ~1.3 ml culture into each well of a 96-deep well plate and harvest cells by centrifugation at 1,500 × g for 5-10 minutes at 4ºC.
2. Remove growth media from the growth block or 96-deep well plate by inverting the block or plate on a stack of paper towels and tap to remove as much liquid as possible.
3. Resuspend the cell pellets in 200 µl Resuspension Buffer with RNase A (described on page 13).
4. Add 200 µl Lysis Buffer to each well.
5. Seal the block or plate with adhesive foil supplied with the kit and mix gently by inverting a few times.
6. Incubate at room temperature for 3 minutes. Do not exceed 5 minutes. Remove the adhesive foil.
7. Add 300 µl of Neutralization/Binding Buffer to each well.
8. Seal the block or plate with a new adhesive foil supplied with the kit and mix gently by inverting a few times. Remove the adhesive foil.
9. Proceed to **Binding DNA**.

Purification Procedure Using Vacuum

Introduction

Instructions are provided below to isolate plasmid DNA using a vacuum manifold. The compatibility of the PureLink™ Plates with various instruments is described on page 14.

Instructions to isolate plasmid DNA using a centrifuge are on page 23.

Materials Needed

- Vacuum manifold and vacuum pump producing a pressure of –600 to –700 millibars (mb) (–450 to –525 mm Hg) *or* automated liquid handling workstation
- PureLink™ HQ 96 Clarification Plate (included in the kit)
- PureLink™ HQ 96 Binding Plate (included in the kit)
- Receiver Plate (included in the kit)
- Wash Buffer with ethanol (page 13)
- Elution Buffer (included in the kit)
- Sterile, distilled water (pH>7.0), **optional**

Follow the recommendations below to improve plasmid yield:

- Pipet the elution buffer or water in the center of the well for proper elution
- Perform a 1 minute incubation with elution buffer
- Use the recommended vacuum pressure
- Increase the elution buffer volume to 150 µl to increase DNA yield (note that the DNA will be diluted)
- Decrease the elution buffer volume to 80 µl to increase the DNA concentration
- Always use sterile water with pH 7-8.5, if you are using water for elution

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Recovery of Elution Volume

Based on the volume of elution buffer used for elution, the recovery of the elution volume will vary and is listed below:

Elution Buffer Volume Used	Recovered Elution Volume
150 µl	~125 µl
100 µl	~75 µl
80 µl	~55 µl

Calibrating Vacuum

We recommend using a vacuum pressure of –600 to –700 millibars (–450 to –525 mm Hg) 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™ Binding Plate on top of the vacuum manifold.
2. Apply vacuum and check the vacuum pressure on the vacuum regulator (usually attached to the manifold or the vacuum pump).
3. Adjust the vacuum pressure on the regulator to obtain the recommended pressure of –600 to –700 mb.

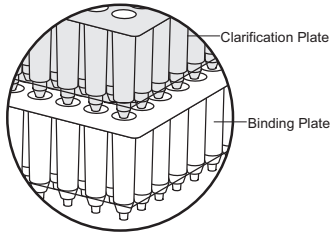
Note: During purification the vacuum pressure may exceed the recommended value.

Continued on next page

Binding DNA

1. Set up the vacuum manifold using manufacturer's recommendations. Use a vacuum pressure of –600 to –700 mb as specified on the previous page.

   Prepare the automated liquid handling workstation deck as recommended by the manufacturer.
2. Place a PureLink™ HQ 96 Binding Plate with long drip director inside the vacuum manifold base. Check the label on the right side of the plate to be sure you are using the Binding Plate.
3. Place a PureLink™ HQ 96 Clarification Plate with large orifice drip director on top of the manifold such that when the vacuum is applied samples will pass through the PureLink™ HQ 96 Clarification Plate and into the PureLink™ HQ 96 Binding Plate (see figure below). Check the label on the right side of the plate to be sure you are using the Clarification Plate.



   Note: If you wish to process less than 96 samples, cover unused wells of the Clarification and Binding Plates with adhesive foil included in the kit.
4. Transfer the lysate (~700 µl) from Step 8, page 18 to the PureLink™ HQ 96 Clarification Plate using a multichannel pipettor or robotic loading device.
5. Apply vacuum for 1 minute. Release the vacuum. Discard the PureLink™ HQ 96 Clarification Plate.
6. Transfer the Binding Plate from the base of the vacuum manifold to the top of the vacuum manifold. Be sure to place waste tray at the base of the manifold to collect the flow through material.
7. Apply vacuum for 1-2 minutes until all samples have passed through the Binding Plate. Release the vacuum.
8. Proceed to **Washing DNA**, next page.

Continued on next page

Purification Procedure Using Vacuum, Continued

Washing DNA

1. Add 600 µl of Wash Buffer prepared with ethanol as described on page 13 to the Binding Plate.
2. Apply vacuum (at –600 to –700 mb as previously specified) for 2 minutes. Release the vacuum. **Repeat** Wash Step one more time.
3. Apply vacuum for 10 minutes to remove any residual Wash Buffer. Release the vacuum.

   To remove any residual liquid trapped in nozzles or bottom of the plate, tap the plate on a stack of paper towels to blot the plate dry. Traces of Wash Buffer may inhibit any downstream enzymatic reactions.
4. Proceed to **Eluting DNA**, below.

Eluting DNA

1. Place the Receiver Plate included in the kit in the vacuum manifold (in place of the waste collection tray) and place the Binding Plate on the manifold.

   Note: To avoid any cross contamination and ensure contact between the PureLink™ HQ 96 Binding Plate and Receiver Plate, raise the Receiver Plate in the vacuum manifold.
2. Add 100 µl Elution Buffer (10 mM Tris-HCl, pH 8.5) or sterile, distilled water (pH >7.0) to the center of the well of the Binding Plate.

   Note: You can use an Elution Buffer volume of 80-150 µl (see page 20).
3. Incubate the plate at room temperature for 1 minute.
4. Apply vacuum for 2-5 minutes to elute the plasmid DNA into the Receiver Plate. Release the vacuum.
5. Store plasmid DNA at -20ºC in the Receiver Plate or transfer plasmid DNA to sterile tubes.
6. Determine the quantity and quality of the isolated plasmid DNA as described on page 25 or proceed to the downstream application of choice.

Purification Procedure Using Centrifugation

Introduction

Instructions are provided below to isolate plasmid DNA using a centrifuge. The compatibility of the PureLink™ Plates with various instruments is described on page 14.

Materials Needed

- Centrifuge with a swinging bucket rotor with plate carriers that have a plate height clearance of ~7.0 cm
- 1.1 ml 96 deep-well plate to collect flow through (cat. no. 12193-025 or equivalent)
- PureLink™ HQ 96 Clarification Plate (included in the kit)
- PureLink™ HQ 96 Binding Plate (included in the kit)
- Receiver Plate (included in the kit)
- Wash Buffer with ethanol (page 13)
- Elution Buffer (included in the kit)
- Sterile, distilled water (pH>7.0)

Binding DNA

Perform all centrifugation steps at room temperature.

1. Place a PureLink™ HQ 96 Clarification Plate with large orifice drip director on a 1.1 ml 96 deep-well collection plate. Check the label on the right side of the plate to be sure you are using the Clarification Plate.

   Note: To process less than 96 samples, cover unused wells of the Clarification and Binding plates with adhesive foil included in the kit
2. Transfer the lysate (~700 µl) from Step 8, page 18 to the PureLink™ HQ 96 Clarification Plate using a multichannel pipettor.
3. Centrifuge the stacked plates at 1,800 × g for 1 minute.
4. Transfer the flow through from the collection plate on to the PureLink™ HQ 96 Binding Plate using a multichannel pipettor. Discard the Clarification Plate.

   You can use the empty collection plate to collect waste during the next centrifugation step.
5. Place the PureLink™ HQ 96 Binding Plate on top of the collection plate from Step 4.
6. Centrifuge the stacked plates at 1,800 × g for 3 minutes to bind the plasmid DNA.
7. Proceed to **Washing DNA**, next page.

Purification Procedure Using Centrifugation, Continued

Washing DNA

1. Add 600 µl Wash Buffer with ethanol (page 13) to the Binding Plate.
2. Centrifuge the stacked plates at 1,800 × g for 3 minutes. Discard flow-through**.**
3. **Repeat** the Wash Step one more time. Discard the flow through.
4. Place the Binding Plate on top of a dry collection plate and centrifuge the stacked plates at 1,800 × g for 5 minutes to remove any residual wash buffer.
5. Proceed to **Eluting DNA**, below.

Eluting DNA

1. Place the PureLink™ HQ 96 Binding Plate on top of a new Receiver Plate included in the kit.
2. Add 100 µl Elution Buffer (10 mM Tris-HCl, pH 8.5) or sterile, distilled water (pH >7.0) to the center of the well of the Binding Plate.

   Note: You can use an Elution Buffer volume of 80-120 µl (page 20).
3. Incubate the plate at room temperature for 1 minute.
4. Centrifuge the stacked plates at 1,800 × g for 5 minutes to elute the plasmid DNA.
5. Store plasmid DNA at -20ºC in the Receiver Plate or transfer plasmid DNA to sterile tubes.
6. Determine the quantity and quality of the isolated plasmid DNA as described on page 25 or proceed to the downstream application of choice.

Plasmid DNA Yield and Quality

Estimating DNA Yield

Plasmid DNA isolated using the PureLink™ HQ 96 Plasmid DNA Purification Kit is easily quantitated using UV absorbance at 260 nm or Quant-iT™ DNA Assay Kits.

UV Absorbance

1. Measure the A_{260} of the solution using a spectrophotometer blanked against 10 mM Tris-HCl, pH 7.5.

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

2. Calculate the amount of DNA using formula:

$$\text{DNA } (\mu g) = A_{260} \times 50\ \mu g/(1\ A_{260} \times 1\ ml) \times \text{dilution factor} \times \text{total sample volume (ml)}$$

For dsDNA, A_{260} = 1 for a 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 (see page viii for ordering information) 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 kit contains 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

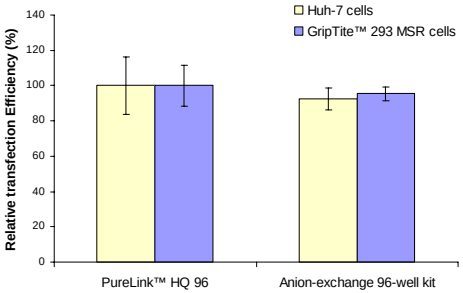
Typically, DNA isolated using the PureLink™ HQ 96 Plasmid DNA Purification Kit has an A_{260}/A_{280} >1.80 when samples are diluted in Tris-HCl (pH 7.5). An A_{260}/A_{280} of >1.80 indicates that the DNA is reasonably clean of proteins that could interfere with downstream applications.

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

Applications

Mammalian Transfection

The plasmid DNA purified using the PureLink™ HQ 96 Plasmid DNA Purification Kit is of high quality and suitable for use in mammalian transfection without the need for alcohol precipitation (see figure below).



Legend

0.3 µg of the plasmid pcDNA™3.1/*lacZ*/His was isolated from TOP10 *E. coli* cells using the PureLink™ HQ 96 Plasmid DNA Purification Kit and a leading competitor's anion exchange 96 well system. The purified plasmid DNA was transfected into GripTite™ 293 MSR and Huh-7 cells using Lipofectamine™ 2000 Reagent. Average transfection efficiencies are expressed as percentages relative to the efficiency obtained with the PureLink™ HQ 96 Plasmid DNA Purification Kit (100%) for each cell line.

Each bar represents the average of 96 samples (each sample in triplicate) ± 1 SD for PureLink™ HQ 96 Purification Kit and the average of 27 samples ± 1 SD for the anion-exchange system.

DNA Sequencing

Template quality and consistency are critical in automated fluorescent DNA sequencing. DNA templates prepared using PureLink™ HQ 96 Plasmid DNA Purification Kit are suitable for use in automated fluorescent sequencing with >98% accuracy of base identity for a 600-base read.

PCR and Restriction Enzyme Digestion

The plasmid DNA is eluted in low salt elution buffer without EDTA eliminating the need for further alcohol precipitation or desalting and can be directly used in restriction enzyme digestion or PCR reactions.

Troubleshooting

Introduction

Review the information below to troubleshoot experiments with the PureLink™ HQ 96 Plasmid DNA Purification Kit.

To troubleshoot problems with the vacuum manifold or automated liquid handling workstation, contact the manufacturer.

Problem	Possible Solution
Low yield	• Check the growth conditions of the cell culture to ensure plasmid propagation. Use a high copy number plasmid. • Use the recommended cell number and volume to obtain efficient cell lysis and clarification. • Ensure complete resuspension of the bacterial cell pellet. • If the cell lysate is too viscous, reduce the amount of cells used per sample. • Be sure the Resuspension Buffer contained RNase (page 13) and the Wash Buffer was diluted with ethanol (page 13) before use. • Check the label on the right side of the plate to make sure the correct plate is used for Binding and Clarification. If you accidentally used the Clarification Plate instead of Binding Plate, the plasmid DNA will not bind and will be in the flow through. • Ensure that DNA elution was performed with a low salt buffer (e.g., the Elution Buffer supplied in the kit) or sterile distilled water (pH >7.0) and perform incubation for 1 minute with elution buffer to obtain the best results.
Poor Plasmid DNA Quality	• Avoid using endA$^+$ bacterial strains if possible. • Ensure the Binding Plate is completely free from any residual Wash Buffer prior to elution. Traces of ethanol from the Wash Buffer can inhibit downstream enzymatic reactions.

Problem	Possible Solution
Genomic DNA contamination	• Perform lysis and neutralization with gentle mixing as described on page 18 to avoid shearing genomic DNA. Intact genomic DNA is efficiently precipitated away from the plasmid DNA. • Avoid using dense cultures. Use the recommended number of cells. • Do not exceed 5 minutes of cell lysis protocol (Step 8, page 18). • When purifying using centrifugation, do not centrifuge longer than 1 minute when binding the DNA (**Binding DNA**, page 23, Step 3).
Low elution volume or sample cross-contamination	• Make sure the vacuum manifold is sealed tightly and there is no leakage. A vacuum pressure of –600 to –700 mb is required to obtain the best results. • To avoid any cross contamination and ensure contact between the PureLink™ HQ 96 Binding Plate and Receiver Plate, raise the Receiver Plate in the vacuum manifold.

Appendix

Technical Support

Technical Resources on the 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).

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MSDS Requests

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

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