

ChargeSwitch®-Pro Filter Plasmid Miniprep Kit

**For purification of plasmid DNA from
bacterial cells**

Catalog nos. CS31102 and CS31103

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MAN0001634

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Kit Contents and Storage

Shipping and Storage

All components are shipped at room temperature and stored at room temperature.

Do not freeze the columns. Freezing may damage the ChargeSwitch®-derivatized membrane in the columns.

All components are guaranteed stable for 6 months when stored properly.

Kit Contents

The components of each ChargeSwitch®-Pro Filter Plasmid Miniprep Kit is listed below:

10 Miniprep purifications (Cat. no. CS31102)

100 Miniprep purifications (Cat. no. CS31103)

Component	Amounts/Kit	
	CS31102	CS31103
ChargeSwitch®-Pro Resuspension Buffer (10 mM Tris-HCl, pH 8.5, 10 mM EDTA)	4 ml	25 ml
ChargeSwitch®-Pro Lysis Buffer	4 ml	25 ml
ChargeSwitch®-Pro Precipitation Buffer	4 ml	25 ml
RNase A	80 µl	800 µl
ChargeSwitch®-Pro Wash Buffer 1	10 ml	75 ml
ChargeSwitch®-Pro Wash Buffer 2	4 ml	25 ml
ChargeSwitch®-Pro Elution Buffer (10 mM Tris-HCl, pH 8.5)	4 ml	10 ml
ChargeSwitch®-Pro Filter Plasmid Miniprep Columns	10	50 × 2
ChargeSwitch®-Pro Elution Tubes	10	50 × 2

Accessory Products

Additional Products

The table below lists additional products available from Invitrogen that may be used with the ChargeSwitch®-Pro Filter Plasmid Miniprep Kit.

A large selection of Invitrogen products is available for cleanup of DNA and RNA from various sources. For more information, visit www.invitrogen.com or contact Technical Support (page 18).

Product	Amount	Catalog no.
EveryPrep™ Universal Vacuum Manifold	1 unit	K211101
Quant-iT™ DNA Assay Kit, High Sensitivity	1000 assays	Q33120
Quant-iT™ DNA Assay Kit, Broad-Range	1000 assays	Q33130
Quant-iT™ PicoGreen® dsDNA Assay	1 kit, 1 ml	P7589
Luria Broth Base (Miller's LB Broth Base)®, powder	2.5 kg	12795-084
Ampicillin	200 mg	11593-019
Carbenicillin, Disodium Salt	5 g	10177-012
One Shot® TOP10 Chemically Competent <i>E. coli</i>	10 reactions	C4040-10
	20 reactions	C4040-03
	40 reactions	C4040-06
One Shot® TOP10 Electrocomp™ <i>E. coli</i>	10 reactions	C4040-50
	20 reactions	C4040-52
ChargeSwitch® PCR Cleanup Kit	100 preps	CS12000
PureLink™ PCR Purification Kit	50 preps	K3100-01
PureLink™ Gel Extraction Kit	50 preps	K2100-12

Introduction

Overview

Introduction

The ChargeSwitch®-Pro Filter Plasmid Miniprep Kit contains all the components required for the rapid and efficient isolation of highly pure plasmid DNA from *E. coli* cells. The purification columns in the kit contain a novel ChargeSwitch®-derivatized membrane that is positively charged at low pH and neutral at pH 8.5, to bind and elute plasmid DNA without the use of harsh reagents.

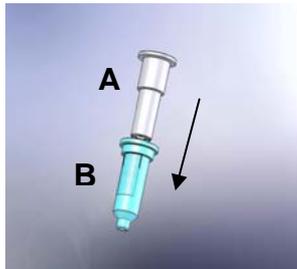
Using the kit, you prepare cell lysates with a modified alkaline lysis procedure and then purify the plasmid DNA using a simple centrifugation- or vacuum-based protocol.

Elute the DNA by raising the pH to 8.5 using a low-salt elution buffer. The purified plasmid DNA is ready for use in your downstream application of choice, including mammalian transfection, automated fluorescent DNA or manual sequencing, PCR, cloning, *in vitro* transcription, bacterial cell transformations, or restriction digestion.

Filter Column Assembly

The ChargeSwitch®-Pro Filter Plasmid Miniprep Kit employs a nested column design consisting of an inner flat bottomed column (A), which fits into an outer luer bottomed column (B). The column assembly is provided in a 2 ml Collection Tube.

The inner column (Lysate Clarification Column) is used for rapid clarification of the bacterial lysate. The outer column (Binding Column) contains the ChargeSwitch® derivitized membrane which binds plasmid DNA from the clarified lysate.



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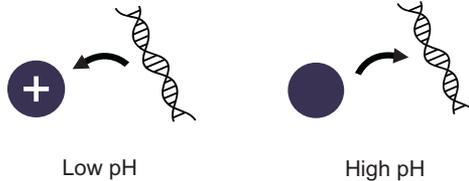
Overview, continued

ChargeSwitch® Technology

ChargeSwitch® Technology provides a switchable surface that is charge dependent on the pH of the surrounding buffer to facilitate nucleic acid purification.

In low pH conditions, the ChargeSwitch® purification membrane has a positive charge that binds the negatively charged nucleic acid backbone. Proteins and other contaminants are not bound and are simply washed away in aqueous wash buffers.

To elute nucleic acids, the charge on the surface is neutralized by raising the pH to 8.5 using a low salt elution buffer. Purified DNA elutes instantly into this elution buffer, and is ready for use in downstream applications of choice.



Advantages of the Kit

The ChargeSwitch®-Pro Filter Plasmid Miniprep Kit offers the following advantages:

- High-quality, high-yield plasmid DNA purification from *E. coli* without the use of ethanol, chaotropic salts, or organic solvents.
 - Designed to isolate plasmid DNA from samples using a simple centrifugation or vacuum protocol following sample preparation.
 - Reliable performance of the purified plasmid DNA in a variety of applications, including mammalian cell transfection, automated and manual sequencing, amplification reactions, *in vitro* transcription, bacterial cell transformation, cloning, and labeling.
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Overview, continued



Note

The ChargeSwitch®-Pro Filter Plasmid Miniprep Kit is compatible for use in isolation of plasmid from *endA*⁺ strains.

System Specifications

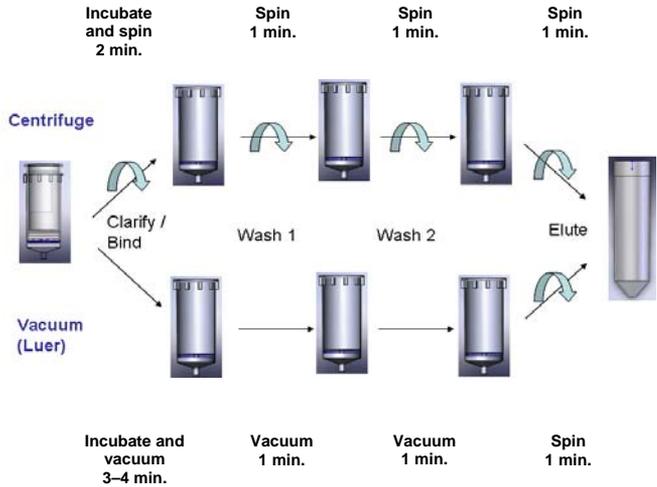
	Miniprep
Starting Material (fresh, overnight LB culture)	5 ml
Binding Capacity (per column)	40 µg
Elution Volume	50–150 µl
Typical DNA Yield	25 µg
Plasmid Size	3–9 kb
Purity OD 260/280	>1.8
Purity OD 260/230	>1.8
Endotoxin (EU/µg DNA)	≤2

Experimental Workflows

Workflow

Samples can be processed by centrifugation or by vacuum manifold. A vacuum manifold allows convenient processing of samples by reducing the number of handling and centrifugation steps. The diagram below show the workflows for the kits using centrifugation (upper), and luer lock vacuum manifold (lower) protocols.

Centrifugation and Vacuum Workflow



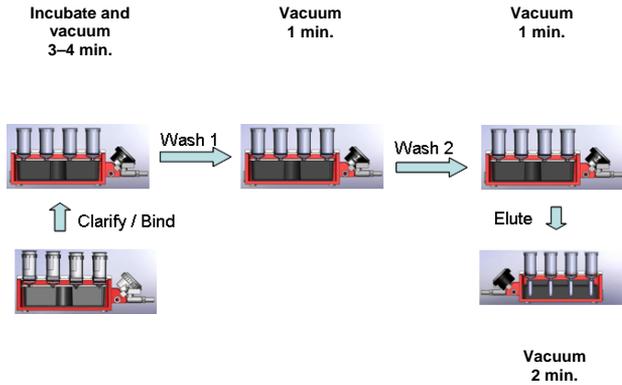
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Experimental Workflows, continued

Workflow

The EveryPrep™ Universal Vacuum Manifold allows convenient processing of samples by reducing the number of handling steps, and supports vacuum assisted elution to eliminate centrifugation altogether. The diagram below shows the workflow for the kits using the EveryPrep™ Universal Vacuum Manifold.

EveryPrep™ Universal Vacuum Manifold Workflow



Methods

General Information

Introduction

Review the information in this section before starting. Guidelines are included for growing the bacterial culture.

Bacterial Cultures

- Grow transformed *E. coli* in LB medium with the appropriate antibiotic. **Do not** use richer medium like Terrific Broth to grow the *E. coli*.
 - Use overnight bacterial cultures with an absorbance of 1–2 OD at 600 nm (A_{600}).
 - Use 5 ml of bacterial culture for minipreps.
 - For best results, use fresh overnight cultures, however, the kit can also be used to purify plasmid DNA from frozen cell pellets.
-

Handling DNA

- Maintain a sterile environment when handling DNA to avoid any contamination from DNases
 - Ensure that no DNase is introduced into the solutions supplied with the kit
 - Make sure that all equipment coming in contact with DNA is sterile, including pipette tips and tubes
 - Store purified plasmid DNA at 4° C for immediate use or at –20° C for long-term storage
 - Avoid repeated freeze-thawing of purified DNA
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Handling the Columns

- **Do not freeze the columns.** Freezing may damage the CST-derivatized membrane.
 - Discard the ChargeSwitch®-Pro Filter Plasmid Columns after use. Columns **cannot** be reused.
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General Information, continued

Elution Buffer

For best results, use the ChargeSwitch®-Pro Elution Buffer provided in the kit. **Do not elute in water.** If it is necessary to elute in another buffer, use a buffer of **pH 8.5–9.0**. If the pH of the buffer is <8.5, the DNA will not elute efficiently.

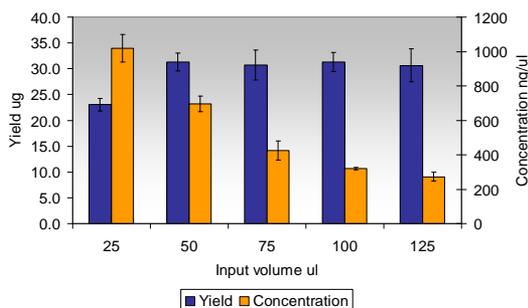
Plasmid DNA is eluted in 50–150 µl of buffer. The volume of elution buffer can be varied to obtain plasmid DNA in the desired final concentration.

For increased DNA yield, use a higher elution buffer volume.

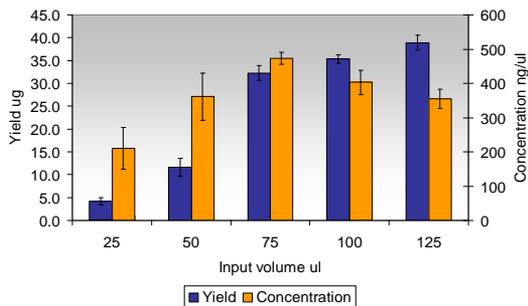
For increased DNA concentration, use a lower elution buffer volume.

The following graphs plots elution volume versus DNA yield and concentration. It is designed to help you determine the most appropriate elution conditions for your application.

Elution Volume versus Yield and Concentration for Centrifuge Protocol



Elution Volume versus Yield and Concentration for Vacuum Protocol



Continued on next page

General Information, continued

Safety Information

Follow the safety guidelines below when using the ChargeSwitch®-Pro Filter Plasmid Miniprep Kit.

- Always wear a suitable lab coat, disposable gloves, and protective goggles.
 - If a spill of the buffers occurs, clean with a suitable laboratory detergent and water. If the liquid spill contains potentially infectious agents, clean the affected area first with laboratory detergent and water, then with 1% (v/v) sodium hypochlorite or a suitable laboratory disinfectant.
-

Miniprep Procedure

Introduction

Protocols for isolating plasmid DNA from up to 5 ml overnight bacterial culture are described in this section.

Materials Needed

In addition to the materials supplied in the kit, you will need the following:

- Up to 5 ml overnight bacterial culture (page 6)
 - Microcentrifuge
 - Vacuum protocol only: Vacuum manifold and vacuum pump (capable of producing pressure of 15–20 in. Hg or –500 to –700 mbar)
Note: Invitrogen produces the EveryPrep™ Universal Vacuum Manifold (see page vi for ordering information), which is ideal for this application.
 - Adjustable pipettes and aerosol barrier pipette tips
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Before Starting

ChargeSwitch®-Pro Resuspension Buffer

Add the entire contents of supplied ChargeSwitch®-Pro RNase A to the ChargeSwitch®-Pro Resuspension Buffer. Mix well. Mark the box on the bottle to indicate that the RNase A has been added. Store the buffer with RNase A at room temperature.

ChargeSwitch®-Pro Lysis Buffer

Check the ChargeSwitch®-Pro Lysis Buffer for precipitates. If necessary, warm the buffer to 37°C to dissolve any precipitate.

ChargeSwitch®-Pro Precipitation Buffer

If room temperature is >25°C, chilling the ChargeSwitch®-Pro Precipitation Buffer on ice before use may improve results.

Continued on next page

Miniprep Procedure, continued

Preparing the Bacterial Lysate

1. Harvest up to 5 ml of overnight bacterial culture by centrifugation at $\geq 8,500 \times g$ for 10 minutes.
2. Resuspend the cell pellet in 250 μl of ChargeSwitch[®]-Pro Resuspension Buffer premixed with RNase A (see **Before Starting**, previous page). Invert the tube or vortex until any remaining cell clumps are dispersed.
3. Add 250 μl of ChargeSwitch[®]-Pro Lysis Buffer. Mix by inverting capped tube 5–10 times until the lysate becomes homogenous. **Do not vortex**, as this may result in shearing of the genomic DNA.
4. Incubate at room temperature for 5 minutes. The lysate will become clear and viscous. **Do not incubate longer than 5 minutes.**
5. Add 250 μl of ChargeSwitch[®]-Pro Precipitation Buffer. Immediately mix by inversion 6–10 times until the precipitate that forms becomes homogeneous. **Do not vortex.**
6. Proceed immediately to **Centrifugation Protocol**, next page, or **Vacuum Protocol**, page 14.

Continued on next page

Miniprep Procedure, continued

Centrifugation Protocol

Follow the procedure below to bind, wash, and elute plasmid DNA using a microcentrifuge. See page 14 for a vacuum-based protocol.

1. Carefully transfer the lysate mixture from Step 5, previous page, onto the ChargeSwitch®-Pro Filter Mini Column in its 2 ml Collection Tube.
 2. Incubate 1 minute at room temperature to allow precipitate to float to the surface.
 3. Centrifuge the column at $1,350 \times g$ for 1 minute.
Note: If the lysate has not completely passed through the column after the initial spin, repeat centrifugation for 1 minute at $8,500 \times g$.
 4. Remove the column assembly and decant the flow-through from the 2 ml Collection Tube.
 5. Remove the inner Lysate Clarification Column from the column assembly (see page 1) and discard. Re-insert the Binding Column into the 2 ml Collection Tube.
 6. Add 750 μ l of ChargeSwitch®-Pro Wash Buffer 1 to the column.
 7. Centrifuge at $8,500 \times g$ for 1 minute.
 8. Remove the column and discard the flow-through from the 2 ml Collection Tube. Re-insert the column into the 2 ml Collection Tube.
 9. Add 250 μ l of ChargeSwitch®-Pro Wash Buffer 2 to the column.
 10. Centrifuge at $8,500 \times g$ for 1 minute.
 11. Remove the column from the tube. Discard the flow-through *and* the 2 ml Collection Tube.
 12. Insert the column into a clean 1.7 ml Elution Tube.
 13. Add 50–150 μ l of ChargeSwitch®-Pro Elution Buffer onto the column and incubate for 1 minute.
 14. Centrifuge at $8,500 \times g$ for 1 minute.
 15. The eluate contains the purified plasmid DNA. Store plasmid DNA at 4°C for immediate use or at -20°C for long-term storage.
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Miniprep Procedure, continued

EveryPrep™ Universal Vacuum Manifold Protocol

Follow the procedure below to bind, wash, and elute plasmid DNA using the EveryPrep™ Universal Vacuum Manifold. Use a vacuum pump capable of producing pressure of 15–20 in. Hg or –500 to –700 mbar. Refer to the manual for the EveryPrep™ Universal Vacuum Manifold for details, and alternative protocols.

1. Discard the 2 ml Collection Tube from the ChargeSwitch®-Pro Filter Mini Column assembly.
2. Place the Waste Tray in one chamber of the manifold.
3. Seat the Mini Elution Top Plate above the Waste Tray and insert the required number of assembled columns firmly into the plate.
4. Block the remaining holes with the provided stoppers.
5. Carefully transfer the supernatant from Step 5, page 10, onto the column.
6. Incubate for 1 minute at room temperature to allow the precipitate to float to the surface.
7. Apply maximum vacuum pressure (15–20 in. Hg) until the liquid has passed through the column (approximately 2–3 minutes).
8. Release the vacuum. Gently remove the inner Lysate Clarification Column (see page 1) and discard.
9. Add 750 µl of ChargeSwitch®-Pro Wash Buffer 1 to the column.
10. Apply maximum vacuum pressure until the liquid has passed through the column (approximately 1 minute). Release the vacuum.
11. Add 250 µl of ChargeSwitch®-Pro Wash Buffer 2 to the column.
12. Apply maximum vacuum pressure until the liquid has passed through the column (approximately 1 minute). Release the vacuum.

Continued on next page

Miniprep Procedure, continued

**EveryPrep™
Universal
Vacuum
Manifold
Protocol,
continued**

13. Place the Elution Rack into the Elution Chamber. The Elution Rack should contain a clean 1.7 ml Elution Tube in each position corresponding to a ChargeSwitch®-Pro Filter Mini Column.
 14. Transfer the Mini Elution Top Plate so that it is positioned over the Elution Chamber.
 15. Add 75–150 µl of ChargeSwitch®-Pro Elution Buffer onto each column and incubate for 1 minute.
 16. Apply a vacuum of 5–10 in. Hg for 2 minutes to allow the elution buffer to completely pass through the column before releasing the vacuum.
 17. Remove the Mini Elution Top Plate, and discard the columns.
 18. The eluate contains the purified plasmid DNA. Store purified plasmid DNA at 4° C for immediate use or at –20° C for long-term storage.
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Miniprep Procedure, continued

Vacuum Protocol

Follow the procedure below to bind, wash, and elute plasmid DNA using a vacuum manifold and pump. Use a vacuum pump capable of producing pressure of 15–20 in. Hg or –500 to –700 mbar. See previous page 11 for a centrifuge-based protocol.

1. Discard the 2 ml Collection Tube from the ChargeSwitch®-Pro Filter Mini Column assembly.
 2. Insert the column assembly (see page 1) into the luer extension of a vacuum manifold.
 3. Carefully transfer the supernatant from Step 5, page 10, onto the column.
 4. Incubate for 1 minute at room temperature to allow the precipitate to float to the surface.
 5. Apply maximum vacuum pressure (15–20 in. Hg) until the liquid has passed through the column (approximately 2–3 minutes).
 6. Release the vacuum. Gently remove and discard the inner Lysate Clarification Column.
 7. Add 750 µl of Wash Buffer 1 to the column.
 8. Apply maximum vacuum pressure until the liquid has passed through the column (approximately 1 minute). Release the vacuum.
 9. Add 250 µl of Wash Buffer 2 to the column.
 10. Apply maximum vacuum pressure until the liquid has passed through the column (approximately 1 minute). Release the vacuum.
 11. Remove the column from the manifold and insert it into a clean 1.7 ml Elution Tube.
 12. Add 50–150 µl of Elution Buffer onto the column and incubate for 1 minute.
 13. Centrifuge at $8,500 \times g$ for 1 minute.
 14. The eluate contains the purified plasmid DNA. Store plasmid DNA at 4° C for immediate use or at –20° C for long-term storage. (see page 1)
-

Analyzing Plasmid DNA Yield and Quality

Plasmid DNA Yield

Perform DNA quantitation using UV absorbance at 260 nm or Quant-iT™ Kits.

UV Absorbance

1. Prepare a dilution of the DNA solution. Mix well. Measure the absorbance at 260 nm (A_{260}) of the dilution in a spectrophotometer (using a cuvette with an optical path length of 1 cm) blanked against the dilution buffer.
2. Calculate the concentration of DNA using the formula:
$$\text{DNA } (\mu\text{g/ml}) = A_{260} \times 50 \times \text{dilution factor}$$
For DNA, $A_{260} = 1$ for a 50 $\mu\text{g/ml}$ solution measured in a cuvette with an optical path length of 1 cm.

Quant-iT™ Kits

Quant-iT™ Kits from Invitrogen provide a rapid, sensitive, and specific fluorescent method for dsDNA quantitation. Each kit contains a state-of-the-art quantitation reagent and a pre-made buffer to allow fluorescent DNA quantitation using standard fluorescent microplate readers/fluorometers or the Qubit™ Quantitation Fluorometer. Visit www.invitrogen.com/naprep for more information.

Note: We recommend using a known quantity of plasmid DNA as a standard when calculating yield using Quant-iT™ Kits. The non-supercoiled DNA standard provided in these kits typically fluoresces more brightly than supercoiled plasmid DNA, which may lead to inaccuracies in quantitation.

Plasmid DNA Quality

Typically, plasmid DNA isolated using the ChargeSwitch®-Pro Filter Plasmid Miniprep Kit have an A_{260}/A_{280} ratio of 1.7–2.0 when samples are diluted in Tris-HCl pH 7.5, indicating that the DNA is free of contaminants that could interfere with downstream applications. Absence of contaminating RNA may be confirmed by agarose gel electrophoresis.

Troubleshooting

Introduction

Refer to the table below to troubleshoot problems that you may encounter when purifying plasmid DNA with the kit.

Problem	Cause	Solution
Low plasmid DNA yield	Poor quality of starting material or incomplete lysis	<ul style="list-style-type: none">• Ensure media is completely removed after cell harvest.• If the cell lysate is too viscous, reduce the amount of cells used per sample. Attempting to lyse too many cells may result in incomplete lysis. See the culture volume recommendations on page 6.• Check the growth conditions of the cell culture to ensure plasmid propagation. Use a high copy number plasmid if possible.• Cell cultures that are overgrown (<i>e.g.</i>, grown >16 hours) may begin to lyse, resulting in reduced yields and contaminating genomic DNA• Ensure complete resuspension of the bacterial cell pellet. Decrease the amount of starting material used.• Chill the Precipitation Buffer on ice before use to improve the precipitation efficiency and plasmid DNA yield.• Mix lysate thoroughly (>10 inversions) to ensure complete lysis.• Increase the incubation time during lysis but do not exceed 5 minutes.
	Elution conditions require optimization	<ul style="list-style-type: none">• If you are using a different buffer for elution, ensure that the pH of the buffer is 8.5–9.0.

Continued on next page

Troubleshooting, continued

Problem	Cause	Solution
Low plasmid DNA yield, continued	ChargeSwitch [®] -derivatized membrane is not functional	Do not freeze the columns. Store the columns at room temperature. Do not re-use the columns.
	Cell cultures are overgrown	Cells grown more than 16 hours may begin to lyse, resulting in reduced yields
	Quantitation is inaccurate: Supercoiled plasmid DNA fluoresces less than the DNA standard provided in quantitation kit	We recommend using plasmid DNA as a standard when calculating yield using a fluorescence-based DNA quantitation kit. Such kits typically provide non-supercoiled DNA as a standard, which fluoresces more brightly than supercoiled plasmid DNA, leading to inaccuracies in quantitation.
Lysate Clarification Column clogging	Too much precipitate	<ul style="list-style-type: none"> • Incubate the lysate for 2–3 minutes to allow the precipitate to float. • Cell culture may be overgrown. See page 6 for details on culturing cells.
Genomic DNA contamination	Genomic DNA sheared during handling	Gently invert the tubes to mix after adding buffers. Do not vortex as it can shear the genomic DNA. To efficiently precipitate the genomic DNA away from the plasmid DNA, the genomic DNA must be intact.
RNA contamination	Insufficient RNase treatment	<ul style="list-style-type: none"> • Ensure RNase A is added to the resuspension buffer. • Add additional RNase A to 100 µl/ml if buffer has been stored for >6 months. • Ensure sufficient mixing after addition of lysis buffer to allow proper RNase A digestion.
Plasmid DNA degradation	Incorrect lysis procedure	Incubate the lysate at room temperature for no longer than 5 minutes, because it might begin to denature the DNA.

Appendix

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).

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Product Qualification

The Certificate of Analysis provides detailed quality control information for each product. Certificates of Analysis are available on our website. Go to www.invitrogen.com/support and search for the Certificate of Analysis by product lot number, which is printed on the box.

MSDS

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

Purchaser Notification

**Limited Use
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No. 5: Invitrogen
Technology**

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Purchaser Notification, continued

Limited Warranty

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