USER GUIDE

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ChargeSwitch®-Pro Plasmid MiniPrep Kit

For purification of plasmid DNA from bacterial cells

Catalog number CS30050 and CS30250

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For Research Use Only. Not for use in diagnostic procedures.

life technologies™

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Kit Contents and Storage

Shipping and Storage

All components are shipped at room temperature and should be 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 Plasmid MiniPrep Kit are listed below. Components are provided for 50 purifications (Cat no. CS30050) or 250 purifications (Cat no. CS30250).

Component	Amounts/Kit	
	CS30050	**CS30250**
ChargeSwitch®-Pro Plasmid Resuspension Buffer (10 mM Tris-HCl, pH 8.5, 10 mM EDTA)	20 mL	100 mL
ChargeSwitch®-Pro Plasmid Lysis Buffer	20 mL	100 mL
ChargeSwitch®-Pro Plasmid Precipitation Buffer	20 mL	100 mL
RNase A	0.4 mL	2 mL
ChargeSwitch®-Pro Plasmid Wash Buffer 1	50 mL	240 mL
ChargeSwitch®-Pro Plasmid Wash Buffer 2	25 mL	100 mL
ChargeSwitch®-Pro Plasmid Elution Buffer (10 mM Tris-HCl, pH 8.5)	6 mL	30 mL
ChargeSwitch®-Pro Plasmid Miniprep Columns	50	50 × 5
ChargeSwitch®-Pro Plasmid Miniprep Collection Tubes	50	50 × 5
ChargeSwitch®-Pro Plasmid Miniprep Elution Tubes	50	50 × 5

Introduction

Overview

Introduction

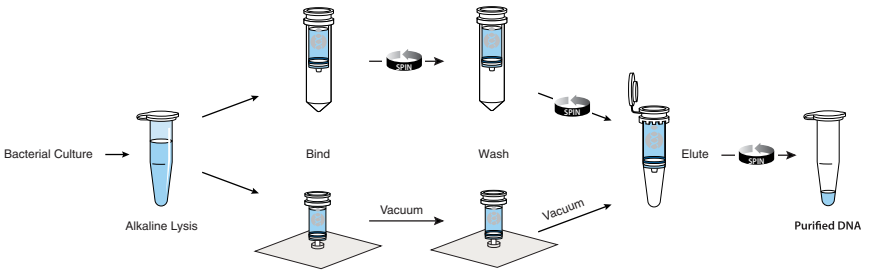
The ChargeSwitch®-Pro Plasmid Miniprep Kit contains all the components required for the rapid and efficient isolation of highly pure plasmid DNA from *E. coli* cells. The purification columns in the kit contain a novel ChargeSwitch®-derivatized membrane that is positively charged at low pH and neutral 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. In low pH conditions, the ChargeSwitch®-derivatized membrane binds the negatively charged nucleic acid backbone. Proteins and other contaminants are not bound and simply wash away in the aqueous wash buffers.

To elute the DNA, the charge of the membrane is neutralized 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.

Workflow

The following diagram shows the centrifugation and vacuum workflows using the kit.



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Advantages of the Kit

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

- High-quality, high-yield (≤20 µg) 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.

Note

The ChargeSwitch®-Pro Plasmid Miniprep Kit is not optimized for use with *endA*+ strains.

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.

System Specifications

Starting Material:	1–5 mL fresh, overnight LB culture
Binding Capacity:	Up to 20 µg plasmid DNA per column
Elution Volume:	25–100 µL
DNA Yield:	≤20 µg

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. If desired, you may use richer medium like Terrific Broth to grow the *E. coli.*
- Use 1–5 mL of overnight bacterial cultures with an absorbance of up to 9 OD at 600 nm (A_{600}).
- For best results, use fresh overnight cultures. 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.

Handling the Columns

- **Do not freeze the columns.** Freezing may damage the CST-derivatized membrane.
- **Do not add oxidizing agents** such as bleach to the column or column flow-through. Do not dispose of columns in bleach.

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Elution Buffer

For best results, use the Elution Buffer provided in the kit. **Do not elute in water**. If you need to elute in any other buffer, be sure to 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 25–100 µL of buffer. The volume of elution buffer can be changed to obtain plasmid DNA in the desired final concentration.

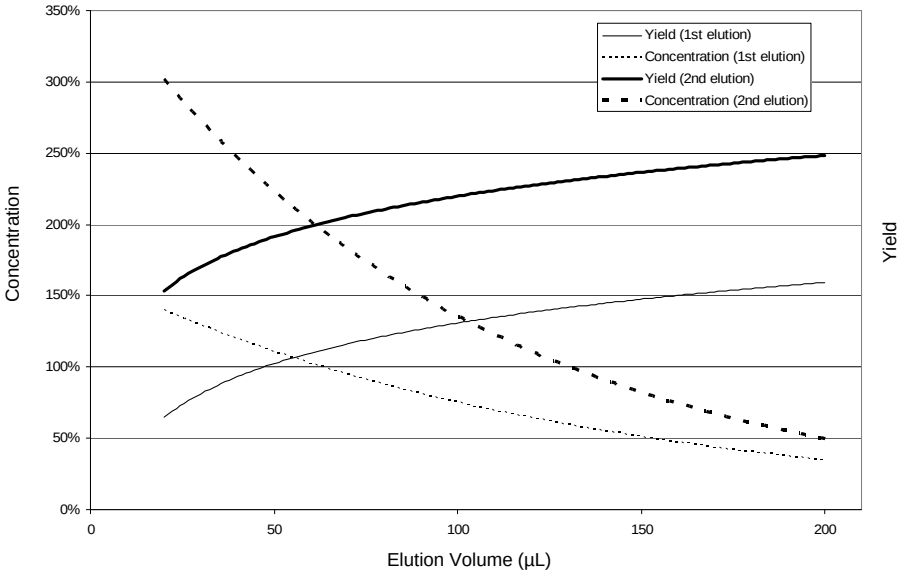
Elution Volume versus Yield and Concentration

The following graph displays trend lines showing elution volume and number of elutions versus DNA yield and concentration. It is designed to help you determine the most appropriate elution conditions for your application.

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

To maximize DNA yield, we recommend transferring the entire volume of eluate from the first elution back onto the column and performing a second elution.

Note: In the graph, yield has been normalized to 100% for a single 50-µL elution.



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Safety Information

Follow the provided safety guidelines when using the ChargeSwitch®-Pro Plasmid MiniPrep Kit.

- Always wear a suitable lab coat, disposable gloves, and protective goggles.
- **Do not** add bleach or oxidizing agents directly to the columns or sample preparation waste.
- 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.

Isolating Plasmid DNA

Introduction

Protocols for isolating plasmid DNA from 1–5 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:

- 1–5 mL overnight bacterial culture (page 7)
- Sterile 2.0-mL microcentrifuge tubes for preparing the lysate
- Microcentrifuge
- Vacuum protocol only: Vacuum manifold and vacuum pump (producing pressure of 13–15 InHg or –800 to –900 mbar)
- Adjustable pipettes and aerosol barrier pipette tips

Before Starting

Resuspension Buffer (R4)
Add the entire contents of supplied RNase A to the 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.

Lysis Buffer
Check the Lysis Buffer for precipitates. If necessary, warm the buffer to 37ºC to dissolve any precipitate.

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

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Isolating Plasmid DNA, Continued

Preparing the Bacterial Lysate

1. Harvest 1–5 mL of overnight bacterial culture by centrifugation.
2. Resuspend the cell pellet in 250 µL of Resuspension Buffer premixed with RNase A (see **Before Starting,** previous page). Pipet up and down to completely resuspend the pellet. No cell clumps should remain.
3. Add 250 µL of Lysis Buffer. Mix by inverting capped tube 6 times until the solution becomes viscous. **Do not vortex,** as this may result in shearing of the genomic DNA.
4. Incubate at room temperature for 2–5 minutes. The solution will become clear and viscous. **Do not incubate longer than 5 minutes.**
5. Add 250 µL of Precipitation Buffer. Immediately mix by inversion until the solution is homogeneous and a cloudy white precipitate is formed.
6. Centrifuge for 10 minutes at maximum speed to pellet the debris.
7. Proceed immediately to **Centrifugation Protocol**, next page, or **Vacuum Protocol**, page 13.

Note

Orienting the column in the same direction in the microcentrifuge during all centrifugation steps may improve yield. This may be accomplished, for example, by orientating the Life Technologies logo, or any other distinguishing mark, to the outside of the microcentrifuge.

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Centrifugation Protocol

Use the following procedure to bind, wash, and elute the DNA using a microcentrifuge. A protocol using a vacuum is provided on the next page.

1. Carefully transfer the supernatant from Step 6, previous page, onto the ChargeSwitch®-Pro Miniprep Column inserted in a Collection Tube (provided in the kit).
2. Centrifuge the column/tube at maximum speed for 30–60 seconds.
3. Remove the column from the tube and discard the flow-through. Re-insert the column in the same Collection Tube.
4. Add 750 µL of Wash Buffer 1 to the column.
5. Centrifuge the column/tube at maximum speed for 30–60 seconds.
6. Remove the column from the tube and discard the flow-through. Re-insert the column in the tube.
7. Add 250 µL of Wash Buffer 2 to the column.
8. Centrifuge the column/tube at maximum speed for 30–60 seconds.
9. Remove the column from the tube. Discard the flow-through *and* the Collection Tube.
10. Insert the column into an Elution Tube (provided in the kit).
11. Add 25–100 µL of Elution Buffer onto the column.
12. Centrifuge the column/tube at maximum speed for 30–60 seconds.
13. **Optional step to maximize DNA yield:** Remove the Elution Tube and transfer the eluate back onto the same column. Re-insert the column in the tube and centrifuge at maximum speed for 30–60 seconds.
14. The eluate contains the purified plasmid DNA.

Store purified DNA at 4°C for immediate use or at –20°C for long-term storage. Avoid repeated freeze-thawing of DNA.

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Vacuum Protocol

Follow the procedure below to bind, wash, and elute the DNA using a vacuum manifold and pump. Use a vacuum capable of producing pressure of 13–15 in. Hg or –800 to –900 mbar. A microcentrifuge protocol is provided on the previous page.

1. Insert the ChargeSwitch®-Pro Miniprep Column into the luer extension of a vacuum manifold.
2. Carefully transfer the supernatant from Step 6, **Preparing the Bacterial Lysate**, onto the column.
3. Apply vacuum pressure until the liquid has passed through the column.
4. Add 750 µL of Wash Buffer 1 to the column.
5. Apply vacuum pressure until the liquid has passed through the column.
6. Add 250 µL of Wash Buffer 2 to the column.
7. Apply vacuum pressure until the liquid has passed through the column.
8. Remove the column from the manifold and insert it into a Collection Tube (provided in the kit).
9. Centrifuge the column/tube at maximum speed for 30–60 seconds.
10. Remove the column from the tube. Discard the flow-through *and* the Collection Tube.
11. Insert the column into an Elution Tube (provided in the kit).
12. Add 25–100 µL of Elution Buffer onto the column.
13. Centrifuge the column/tube at maximum speed for 30–60 seconds.
14. **Optional step to maximize DNA yield:** Remove the Elution Tube and transfer the eluate back onto the same column. Re-insert the column in the tube and centrifuge at maximum speed for 30–60 seconds.
15. The eluate contains the purified plasmid DNA.

Store purified DNA at 4°C for immediate use or at –20°C for long-term storage. Avoid repeated freeze-thawing of DNA.

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 following formula:

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

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

Quant-iT™ Kits

Quant-iT™ Kits from Life Technologies 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.lifetechnologies.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 Plasmid Miniprep Kit has 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 following table 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	• 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 7. • 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 (e.g., 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. • Increase the incubation time during lysis but do not exceed 5 minutes.
	Centrifugation conditions require optimization	Orienting the columns in the same direction in the microcentrifuge during all centrifugation steps may improve yield.
	Elution conditions require optimization	• Perform the optional double-elution step (Step 4, **Eluting the DNA**), by pouring the elution volume back onto the same column. • If you are using a different buffer for elution, ensure that the pH of the buffer is 8.5–9.0.

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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.
Genomic DNA contamination	Genomic DNA sheared during handling	Gently invert 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.
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

Accessory Products

Additional Products

The following table lists additional products available from Life Technologies that may be used with the ChargeSwitch®-Pro Plasmid Miniprep Kit.

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

Product	Amount	Catalog No.
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 *E. coli*	10 reactions	C4040-10
	20 reactions	C4040-03
	40 reactions	C4040-06
One Shot® TOP10 Electrocomp™ *E. coli*	10 reactions	C4040-50
	20 reactions	C4040-52
PureLink® HiPure Filter Maxiprep Kit	25 preps	K2100-16
ChargeSwitch® PCR Cleanup Kit	100 preps	CS12000
PureLink® PCR Purification Kit	50 preps	K3100-01
PureLink® Quick Gel Extraction Kit	50 preps	K2100-12

Technical Support

Obtaining Support

For the latest services and support information for all locations, go to **www.lifetechnologies.com**.

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support (**techsupport@lifetech.com)**
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

Safety Data Sheets (SDS)

Safety Data Sheets (SDSs) are available at **www.lifetechnologies.com/support.**

Certificate of Analysis

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

Limited Product Warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

Purchaser Notification

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The purchase of this product conveys to the purchaser the limited, non-transferable right to use the purchased amount of the product only to perform internal research for the sole benefit of the purchaser. No right to resell this product or any of its components is conveyed expressly, by implication, or by estoppel. This product is for internal research purposes only and is not for use in commercial applications of any kind, including, without limitation, quality control and commercial services such as reporting the results of purchaser's activities for a fee or other form of consideration. For information on obtaining additional rights, please contact **outlicensing@lifetech.com** or Out Licensing, Life Technologies, 5791 Van Allen Way, Carlsbad, California 92008.

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