invitrogen™

ChargeSwitch®-Pro Plasmid Miniprep Kit

Cat. no. CS30010 **Size: 10 reactions**

Store at room temperature

Description

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.

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

- High-quality, high-yield (up to 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.

Specifications

Starting Material:	1–5 ml fresh, overnight LB culture
Binding Capacity:	Up to 20 µg plasmid DNA per column
Elution Volume:	50–100 µl
DNA Yield:	Up to 20 µg

Component	Quantity
ChargeSwitch®-Pro Plasmid Resuspension Buffer (10 mM Tris-HCl, pH 8.5, 10 mM EDTA)	4 ml
RNase A	80 µl
ChargeSwitch®-Pro Plasmid Lysis Buffer	4 ml
ChargeSwitch®-Pro Plasmid Precipitation Buffer	4 ml
ChargeSwitch®-Pro Plasmid Wash Buffer 1	10 ml
ChargeSwitch®-Pro Plasmid Wash Buffer 2	4 ml
ChargeSwitch®-Pro Plasmid Elution Buffer (10 mM Tris-HCl, pH 8.5)	4 ml
ChargeSwitch®-Pro Plasmid Miniprep Columns	10
ChargeSwitch®-Pro Plasmid Miniprep Collection Tubes	10
ChargeSwitch®-Pro Plasmid Miniprep Elution Tubes	10

Shipping and Storage

All components are shipped and should be stored at room temperature. **Do not freeze the columns.**

Additional Products

Product	Amount	Catalog No.
ChargeSwitch®-Pro Plasmid Miniprep Kit	50 reactions	CS30050
	250 reactions	CS30250

Part no. CS30010.pps Rev. date: 14 Sep 2006

Important Guidelines and Parameters

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.

Buffers

- For a new kit, add the entire volume of RNase A provided in the kit to the Resuspension Buffer and mix.
- If necessary, warm the Lysis Buffer to 37ºC to dissolve any precipitate.
- If room temperature is >25°C, chilling the Precipitation Buffer on ice before use may improve results.
- 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.

Columns

- Orienting the columns in the same direction in the microcentrifuge during all centrifugation steps may improve yield.
- **Do not freeze the columns.** Freezing will 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.

Centrifugation Protocol

Follow the steps below to purify plasmid DNA from 1–5 ml of fresh overnight cultures using a microcentrifuge. All steps are performed at room temperature.

Before starting: Add the entire volume of RNase A to the Resuspension Buffer and mix before first use. If necessary, warm the Lysis Buffer to 37ºC to dissolve any precipitate.

Preparing the Sample

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,** above). Pipet up and down to completely resuspend the pellet. No cell clumps should remain.
3. Add 250 µl of Lysis Buffer. Mix by inverting the capped tube 6 times until the solution becomes viscous. Do not vortex.
4. Incubate at room temperature for 2–5 minutes. **Do not incubate longer than 5 minutes.**
5. Add 250 µl of Precipitation Buffer. Mix by inversion until the solution is homogeneous and a white precipitate is formed.
6. Centrifuge for 10 minutes at maximum speed to pellet the debris.

Binding the DNA

1. Carefully transfer the supernatant onto the ChargeSwitch®-Pro Miniprep Column inserted in a Collection Tube.
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.

Washing the Column

1. Add 750 µl of Wash Buffer 1 to the column.
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 tube.
4. Add 250 µl of Wash Buffer 2 to the column.
5. Centrifuge the column/tube at maximum speed for 30–60 seconds.
6. Remove the column from the tube. Discard the flow-through *and* the Collection Tube.

Eluting the DNA

1. Insert the column into an Elution Tube (provided in the kit).
2. Add 50–100 µl of Elution Buffer onto the column.
3. Centrifuge the column/tube at maximum speed for 30-60 seconds.
4. **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.
5. The eluate contains the purified plasmid DNA. Store purified DNA at 4°C for immediate use or at –20°C for long-term storage. Calculate DNA yield by UV absorbance at 260 nm.

Vacuum Protocol

Follow the steps below to purify plasmid DNA from 1–5 ml of fresh overnight cultures using a vacuum manifold. All steps are performed at room temperature.

Before starting: Add the entire volume of RNase A to the Resuspension Buffer and mix before first use. If necessary, warm the Lysis Buffer to 37ºC to dissolve any precipitate. If the room temperature is >25°C, chill the Precipitation Buffer on ice before use.

Preparing the Sample

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,** above). Pipet up and down to completely resuspend the pellet. No cell clumps should remain.
3. Add 250 µl of Lysis Buffer. Mix well by inverting the capped tube 6 times until the solution becomes viscous. Do not vortex.
4. Incubate at room temperature for 2–5 minutes. **Do not incubate longer than 5 minutes.**
5. Add 250 µl of Precipitation Buffer. Mix by inversion until the solution is homogeneous and a white precipitate is formed.
6. Centrifuge for 10 minutes at maximum speed to pellet the debris.

Binding the DNA

1. Insert the ChargeSwitch®-Pro Miniprep Column into the luer extension of a vacuum manifold.
2. Carefully transfer the supernatant from Step 6 above onto the column.
3. Apply vacuum pressure until the liquid has passed through the column.

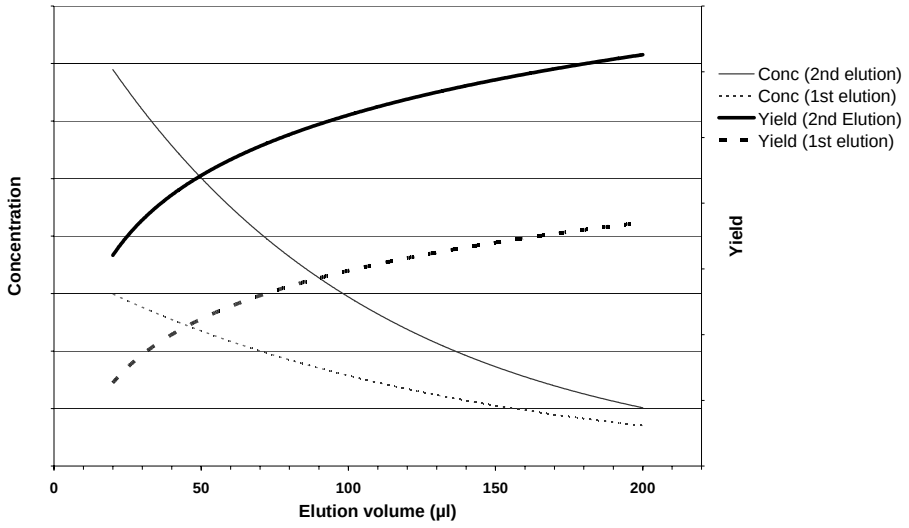
Washing the Column

1. Add 750 µl of Wash Buffer 1 to the column.
2. Apply vacuum pressure until the liquid has passed through the column.
3. Add 250 µl of Wash Buffer 2 to the column.
4. Apply vacuum pressure until the liquid has passed through the column.
5. Remove the column from the manifold and insert it into a Collection Tube (provided in the kit).
6. Centrifuge the column/tube at maximum speed for 30–60 seconds.
7. Remove the column from the tube. Discard the flow-through *and* the Collection Tube.

Eluting the DNA

1. Insert the column into an Elution Tube (provided in the kit).
2. Add 50–100 µl of Elution Buffer onto the column.
3. Centrifuge the column/tube at maximum speed for 30–60 seconds.
4. **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.
5. The eluate contains the purified plasmid DNA. Store purified DNA at 4°C for immediate use or at –20°C for long-term storage. Calculate DNA yield by UV absorbance at 260 nm.

Number and Volume of Elutions Versus DNA Concentration and Percent Recovery



Troubleshooting

Problem	Possible Cause	Solution
Low plasmid DNA yield	Poor quality of starting material or incomplete lysis	• 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 • If the cell lysate is too viscous, reduce the amount of cells used per sample. • Ensure complete resuspension of the bacterial cell pellet. Decrease the amount of starting material used. • Chill the Precipitation Buffer to 4°C 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.
	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.

Product Qualification

This product is qualified in a standard purification procedure from a 5-ml cell culture using the reagents and methods described in this manual. The purified plasmid DNA is analyzed by gel electrophoresis for size and the absence of genomic DNA and RNA contamination. The quantity of purified plasmid DNA is determined using Quant-iT™ Picogreen® dsDNA reagent.

Purchaser Notification

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