invitrogen™

ChargeSwitch®-Pro Filter Plasmid Midi/Maxi Sample Kit

Cat. no. CS31100

Size: 2 Midiprep and 2 Maxiprep reactions
Store at room temperature

Description

The ChargeSwitch®-Pro Filter Plasmid Midi/Maxi Sample 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 Midi/Maxi Sample 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.
- Compatible for use in isolation of plasmid from *endA*+ strains.

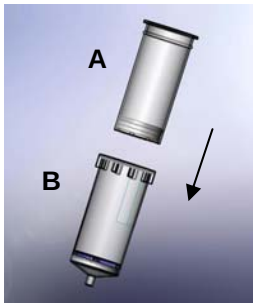
Kit Contents

Component	Quantity
ChargeSwitch®-Pro Resuspension Buffer (10 mM Tris-HCl, pH 8.5, 10 mM EDTA)	25 ml
ChargeSwitch®-Pro RNase A	450 µl
ChargeSwitch®-Pro Lysis Buffer	25 ml
ChargeSwitch®-Pro Precipitation Buffer	25 ml
ChargeSwitch®-Pro Wash Buffer 1	60 ml
ChargeSwitch®-Pro Wash Buffer 2	60 ml
ChargeSwitch®-Pro Elution Buffer (10 mM Tris-HCl, pH 8.5)	10 ml
ChargeSwitch®-Pro Filter Plasmid Midiprep Columns	2
ChargeSwitch®-Pro Filter Plasmid Maxiprep Columns	2

Specifications

The ChargeSwitch®-Pro Filter Plasmid Midi/Maxi Sample Kit employs a nested column design consisting of an inner flat bottomed column (A), which fits into an outer luer bottomed column (B). 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.

	Midiprep	Maxiprep
Starting Material (fresh, overnight LB culture)	25 ml	100 ml
Binding Capacity (per column)	300 µg	1 mg
Recommended Elution Volume	0.5–1 ml	1–2 ml
Typical DNA Yield	200 µg	800 µg
Plasmid Size	3–9 kb	3–9 kb
Purity OD 260/280	>1.8	>1.8
Purity OD 260/230	>1.8	>1.8
Endotoxin	≤1 EU/µg DNA	≤1 EU/µg DNA



Shipping and Storage

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

Part no. 100006307

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Important Guidelines and Parameters

Bacterial Cultures

- Grow transformed *E. coli* in LB medium with the appropriate antibiotic.
- **Do not** use rich medium (e.g., Terrific Broth) to grow the *E. coli.*
- Use overnight bacterial cultures with an absorbance of 1–2 OD at 600 nm (A_{600}).
- For best results, use fresh overnight cultures. However, the kit can also be used to purify DNA from frozen cell pellets.
- Use up to 25 ml of bacterial culture for midipreps, and up to 100 ml of bacterial culture for maxipreps.

Handling DNA

- Maintain a sterile environment and use sterile equipment when handling DNA to avoid DNase contamination.
- Ensure that no DNase is introduced into the solutions supplied with the kit.

Columns

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

Before Starting

- For a new kit, add the entire volume of the provided ChargeSwitch®-Pro RNase A to the ChargeSwitch®-Pro Resuspension Buffer and mix.
- If necessary, warm the Lysis Buffer to 37ºC to dissolve any precipitate.
- If the ambient temperature is >25°C, chill the ChargeSwitch®-Pro Precipitation Buffer on ice before use to 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.

Materials Needed

- Overnight bacterial culture (see **Bacterial Cultures**, above)
- Sterile 50 ml centrifuge tubes (Invitrogen Cat. no. CS32000, or BD Falcon™ Cat. no. 352070)
- Swinging bucket centrifuge
- Vacuum protocol only: Vacuum manifold and pump (capable of producing pressure of 15–20 in. Hg or –500 to –700 mbar). Invitrogen produces the EveryPrep™ Universal Vacuum Manifold (Invitrogen Cat. no. K211101), which is ideal for this application.
- Adjustable pipettes and aerosol barrier pipette tips

Preparing the Sample

1. Harvest the overnight bacterial culture by centrifugation at >2,250 × g for 10 minutes. See below for appropriate volumes.

   Midiprep: ≤25 ml Maxiprep: ≤100 ml

2. Resuspend the cell pellet in ChargeSwitch®-Pro Resuspension Buffer premixed with RNase A (see **Before Starting,** above) using the volumes listed below. Invert the tube or vortex until all cell clumps are dispersed.

   Midiprep: 5 ml Maxiprep: 7 ml

3. Add ChargeSwitch®-Pro Lysis Buffer using the volumes listed below. Mix by inverting the capped tube 10 times until the solution becomes homogenous. **Do not vortex**.

   Midiprep: 5 ml Maxiprep: 7 ml

4. Incubate at room temperature for 5 minutes. The lysate turns clear and viscous. **Do not incubate longer than 5 minutes.**
5. Add ChargeSwitch®-Pro Precipitation Buffer using the volumes listed below. Mix by inversion 6–10 times until the precipitate that forms becomes homogeneous. **Do not vortex**.

   Midiprep: 5 ml Maxiprep: 7 ml

6. Proceed to **Centrifugation Protocol** or **Vacuum Protocol**.

Centrifugation Protocol

Follow the steps below to purify plasmid DNA from overnight cultures using a swinging bucket centrifuge. All steps are performed at room temperature.

Binding the DNA

1. Place the assembled ChargeSwitch®-Pro Filter Midi or Maxi Column into a 50 ml conical tube (not provided).
2. Transfer the lysate (**Preparing the Sample**, Step 5) onto the column.
3. Incubate for 2–3 minutes at room temperature to allow precipitate to float to the surface.
4. Centrifuge the column at >2,250 × g for 2–3 minutes. Gently remove the column assembly and decant the flow-through.
5. Remove and discard the inner Lysate Clarification Column (see page 1). Re-insert the Binding Column into the 50 ml conical tube.

Washing the Column

1. Add 15 ml of ChargeSwitch®-Pro Wash Buffer 1 to the column.
2. Centrifuge at >2,250 × g for 1 minute. Remove the column and discard the flow-through. Re-insert the column into the tube.
3. Add 15 ml of ChargeSwitch®-Pro Wash Buffer 2 to the column.
4. Centrifuge at >2,250 × g for 1 minute. Remove the column. Discard the flow-through *and* the tube.
5. Insert the column into a clean 50 ml conical tube, and proceed to **Eluting the DNA**, below.

Vacuum Protocol

Follow the steps below to purify plasmid DNA from overnight cultures using a luer lock vacuum manifold, pump, and swinging bucket centrifuge. All steps are performed at room temperature. For a vacuum assisted elution protocol, see the manual for the ChargeSwitch®-Pro Filter Plasmid Midiprep and Maxiprep Kit or the EveryPrep™ Universal Vacuum Manifold.

Binding the DNA

1. Insert the ChargeSwitch®-Pro Filter Midi or Maxi Column into the luer extension of a vacuum manifold.
2. Transfer the lysate (**Preparing the Sample**, Step 5) onto the column.
3. Apply maximum vacuum pressure (15–20 in. Hg) until the liquid has passed through the column.
4. Release the vacuum. Gently remove the inner Lysate Clarification Column (see page 1) and discard.

Washing the Column

1. Add 15 ml of ChargeSwitch®-Pro Wash Buffer 1 to the column.
2. Apply maximum vacuum pressure until the liquid has passed through the column. Release the vacuum.
3. Add 15 ml of ChargeSwitch®-Pro Wash Buffer 2 to the column.
4. Apply maximum vacuum pressure until the liquid has passed through the column.
5. Remove the column from the manifold and insert it into a clean 50 ml conical tube. Proceed to **Eluting the DNA**, below.

Eluting the DNA

1. Add ChargeSwitch®-Pro Elution Buffer using the volumes listed below onto the column and incubate for 1 minute.

   Midiprep: 0.5–1 ml Maxiprep: 1–2 ml

2. Centrifuge at >2,250 × g in a swinging bucket centrifuge for 1 minute.
3. Transfer the eluate back onto the same Binding Column and replace it in the same 50 ml conical tube. Centrifuge at >2,250 × g for 1 minute. Discard the Binding Column. **Do not** reuse.
4. The eluate contains the purified plasmid DNA. Store plasmid DNA at 4° C for immediate use or at –20° C for long-term storage. Avoid repeated freeze-thawing of purified DNA. Calculate DNA yield by UV absorbance at 260 nm.

Troubleshooting

Problem	Possible Cause	Solution
Low plasmid DNA yield	Poor quality of starting material or incomplete lysis	• 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 2. • 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 to 4°C 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	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.
Lysate Clarification Column clogging	Too much precipitate	• Incubate lysate for 2–3 minutes to allow precipitate to float. • Cell culture may be overgrown. See page 2 for details on culturing cells.
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.
RNA contamination	Insufficient RNase treatment	• Ensure RNase A is added to the resuspension buffer. • Add additional RNAse A to 100 µl/ml if buffer was stored for >6 months. • Mix sufficiently after addition of lysis buffer for adequate 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.

Additional Products

Product	Amount	Catalog No.
EveryPrep™ Universal Vacuum Manifold	1 unit	K211101
ChargeSwitch®-Pro Filter Collection Tubes	25 tubes	CS32000
ChargeSwitch®-Pro Filter Plasmid Midiprep Kit	25 reactions	CS31104
ChargeSwitch®-Pro Filter Plasmid Maxiprep Kit	10 reactions	CS31106
	25 reactions	CS31107
ChargeSwitch®-Pro Filter Plasmid Miniprep Kit	10 reactions	CS31102
	100 reactions	CS31103

Purchaser Notification

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