invitrogen™

ChargeSwitch®-Pro PCR Cleanup Kit

For purification of PCR products

Catalog no. CS32050 and CS32250

Version A
27 August 2007
25-0984

User Manual

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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 PCR Cleanup Kit are listed below. Components are provided for 50 preps (catalog no. CS32050) or 250 preps (catalog no. CS32250).

Component	Amounts/Kit	
	CS32050	**CS32250**
ChargeSwitch®-Pro PCR Purification Buffer	2.5 ml	12.5 ml
ChargeSwitch®-Pro PCR Wash Buffer	25 ml	125 ml
ChargeSwitch®-Pro PCR Elution Buffer (10 mM Tris-HCl, pH 8.5)	2.5 ml	12.5 ml
ChargeSwitch®-Pro PCR Cleanup Columns, preinserted in Collection Tubes	50	50 × 5
ChargeSwitch®-Pro PCR Elution Tubes	50	50 × 5

Accessory Products

Additional Products

The table below lists related products available from Invitrogen.

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 10).

Product	Amount	Catalog no.
ChargeSwitch® PCR Cleanup Kit	100 preps 960 preps	CS12000 CS12000-10
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
PureLink™ PCR Purification Kit	50 preps	K3100-01
PureLink™ Quick Gel Extraction Kit	50 preps 250 preps	K2100-12 K2100-25

E-Gel® Agarose Gels

E-Gel® Agarose Gels are bufferless pre-cast agarose gels designed for fast, convenient electrophoresis of DNA samples. E-Gel® agarose gels are available in different agarose percentages and well formats. A large variety of DNA ladders is available from Invitrogen for sizing DNA.

For more information, visit www.invitrogen.com or contact Technical Support (page 10).

Introduction

Overview

Introduction

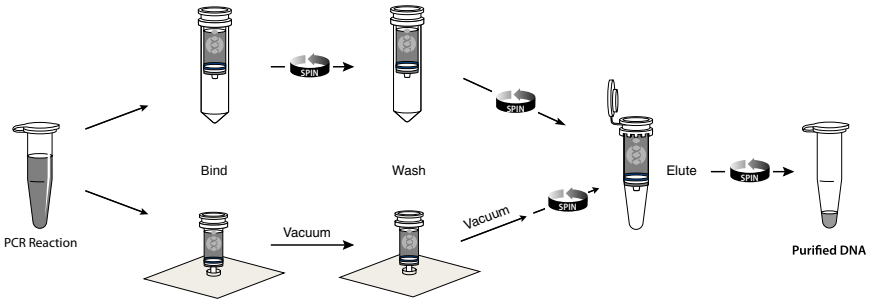
The ChargeSwitch®-Pro PCR Clean-up Kit contains all the components required for the rapid and efficient purification of PCR fragments from the salts, primers, dNTPs, and other non-nucleic acid reagents in a PCR reaction.

The purification columns in the kit contain a novel ChargeSwitch®-derivatized membrane that is positively charged at low pH and neutral at pH 8.5–9.0, to bind and elute PCR products without the use of harsh reagents.

The kit is designed for the purification of PCR fragments ranging in size from 125 bp to 12 kb 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 PCR fragments, the charge of the membrane is neutralized by raising the pH to 8.5–9.0 using a low-salt elution buffer. The purified PCR product is suitable for any downstream applications of choice.

Workflow

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



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Overview, continued

Advantages of the Kit

The ChargeSwitch®-Pro PCR Clean-up Kit offers the following advantages:

- High-quality, high-yield purification of PCR fragments without the use of ethanol, chaotropic salts, or organic solvents.
- Simple, fast centrifugation or vacuum protocol.
- Reliable performance of the purified DNA in a variety of applications, including sequencing, restriction digestion, and cloning.

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 8.5–9.0 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:	25–250 µl PCR reaction
PCR Fragment Size:	125 bp–12 kb
Elution Volume:	50 µl
Purity ($A_{260/280}$):	1.8–2.0

Methods

General Information

Introduction

Review the information in this section before starting.

Important

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.

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

Safety Guidelines

Follow standard laboratory safety guidelines when using the ChargeSwitch® kit:

- Treat all reagents supplied in the kit as potential irritants.
- Always wear a suitable lab coat, disposable gloves, and protective goggles.
- If a buffer spill 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.

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.

Isolating PCR Products—Centrifugation Protocol

Introduction

A protocol for isolating PCR products using a microcentrifuge is provided in this section. A protocol using a vacuum manifold and pump is provided on page 6.

Materials Needed

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

- PCR reaction
- Microcentrifuge
- Adjustable pipettes and aerosol barrier pipette tips

Centrifugation Protocol

Follow the steps below to purify PCR fragments using a microcentrifuge. All steps are performed at room temperature.

Note: After PCR cycling, cool the reaction to room temperature before purification.

Binding the DNA

1. To the PCR reaction, add an equal volume of ChargeSwitch®-Pro PCR Purification Buffer (*e.g.,* for a 50-µl PCR reaction, add 50 µl of Purification Buffer). Briefly vortex to mix.
2. Transfer the mixture onto the ChargeSwitch®-Pro PCR Clean-up Column inserted in a Collection Tube.
3. Centrifuge the column/tube at 10,000 × *g* for 30–60 seconds.
4. Proceed to **Washing the Column**. (**Note:** If the volume of the PCR reaction is >75 µl, empty Collection Tube before proceeding to avoid overflow.)

Protocol continued on next page

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Isolating PCR Products—Centrifugation Protocol, continued

Centrifugation Protocol, continued

Protocol continued from previous page

Washing the Column

1. Add 500 µl of ChargeSwitch®-Pro PCR Wash Buffer to the column.
2. Centrifuge the column/tube at 10,000 × *g* for 1 minute.
3. Discard the flow-through *and* the Collection Tube.
4. Insert the column into a new, sterile Elution Tube (provided in the kit). Then proceed to **Eluting the DNA**.

Eluting the DNA

1. Add 50 µl of ChargeSwitch®-Pro PCR Elution Buffer onto the column, and incubate at room temperature for 1 minute. **Note:** Sample may be eluted in as little as 30 µl of buffer if desired.
2. Centrifuge the column/tube at 10,000 × *g* for 30–60 seconds. **The flow-through contains the purified DNA.**
3. Store the purified DNA at 4°C for immediate use or at –20°C for long-term storage. Calculate DNA yield by Quant-iT™ DNA assay or UV absorbance at 260 nm.

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Isolating PCR Products—Vacuum Protocol

Introduction

A protocol for isolating PCR products using a vacuum manifold and pump is provided in this section. A protocol using a microcentrifuge is provided on page 4.

Materials Needed

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

- PCR reaction
- Microcentrifuge
- Vacuum manifold and vacuum pump (producing pressure of 13–15 in. Hg or –450 to –550 mbar)
- Adjustable pipettes and aerosol barrier pipette tips

Vacuum Protocol

Follow the steps below to purify PCR fragments using a vacuum manifold and pump. All steps are performed at room temperature.

Note: After PCR cycling, cool the reaction to room temperature before purification.

Binding the DNA

1. To the PCR reaction, add an equal volume of ChargeSwitch®-Pro PCR Purification Buffer (*e.g.,* for a 50-µl PCR reaction, add 50 µl of Purification Buffer). Briefly vortex to mix.
2. Remove the ChargeSwitch®-Pro PCR Clean-up Column from the Collection Tube and insert it into the luer extension of a vacuum manifold.
3. Transfer the mixture from Step 1 onto the column.
4. Apply vacuum pressure (13–15 in. Hg or –450 to –550 mbar) until the liquid has passed through the column. Then proceed to **Washing the Column**.

Protocol continued on next page

Isolating PCR Products—Vacuum Protocol, continued

Vacuum Protocol, continued

Protocol continued from previous page

Washing the Column

1. Add 500 µl of ChargeSwitch®-Pro PCR Wash Buffer to the column.
2. Apply vacuum pressure until the liquid has passed through the column.
3. Remove the column from the vacuum manifold and re-insert it into the Collection Tube.
4. Centrifuge the column/tube at 10,000 × *g* for 1 minute to remove any residual liquid.
5. Discard the flow-through *and* the Collection Tube.
6. Insert the column into a new, sterile Elution Tube (provided in the kit). Then proceed to **Eluting the DNA**.

Eluting the DNA

1. Add 50 µl of ChargeSwitch®-Pro PCR Elution Buffer onto the column, and incubate at room temperature for 1 minute. **Note:** Sample may be eluted in as little as 30 µl of buffer if desired.
2. Centrifuge the column/tube at 10,000 × *g* for 1 minute. **The flow-through contains your purified DNA.**
3. Store the purified DNA at 4°C for immediate use or at –20°C for long-term storage. Calculate DNA yield by Quant-iT™ DNA assay or UV absorbance at 260 nm.

Analyzing Yield and Quality

Determining Yield

The quantity of purified DNA may be determined by a Quant-iT™ DNA assay or UV absorbance at 260 nm.

Quant-iT™ Kits

Quant-iT™ DNA assays 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.

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:

   DNA (µg/ml) = A_{260} × 50 × 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.

Determining Quality

Typically, PCR products isolated using the ChargeSwitch®-Pro PCR Cleanup Kit have an A_{260}/A_{280} ratio of 1.8–2.0 when samples are diluted in Tris-HCl pH 8.5, indicating that the DNA is free of contaminants that could interfere with downstream applications.

Troubleshooting and Product Qualification

Introduction

Refer to the table below to troubleshoot problems that you may encounter when isolating PCR products with the kit.

Problem	Cause	Solution
Low yield	Different elution buffer used	If you are using a different buffer for elution, ensure that the pH of the buffer is 8.5–9.0.
	ChargeSwitch®-derivatized membrane has been damaged	Repeat the purification procedure using a new ChargeSwitch®-Pro PCR Purification column. Membrane may be damaged if frozen. Store the columns at room temperature. Do not re-use the columns.

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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MSDS

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

Product Qualification

Product qualification is described in the Certificate of Analysis (CofA), available on our website by product lot number at www.invitrogen.com/cofa.

Purchaser Notification

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Purchaser Notification, continued

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