invitrogen™

ChargeSwitch®-Pro PCR Clean-up Kit

Cat. No. CS32010 **Size: 10 preps**

Store at room temperature

Description

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.

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
- Designed to isolate PCR fragments from reaction mixtures using a simple centrifugation or vacuum protocol
- Reliable performance of the purified DNA in a variety of applications, including sequencing, restriction digestion, and cloning

The ChargeSwitch®-Pro PCR Clean-up Kit is also available in sizes of 50 preps (catalog no. CS32050) and 250 preps (catalog no. CS32050).

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

Required Materials

The following materials are supplied by the user:

- PCR reaction
- Microcentrifuge
- For the vacuum protocol: Vacuum manifold and pump (producing pressure of 13–15 in. Hg or –450 to –550 mbar)

Components

	Quantity
ChargeSwitch®-Pro PCR Purification Buffer	2.5 ml
ChargeSwitch®-Pro PCR Wash Buffer	25 ml
ChargeSwitch®-Pro PCR Elution Buffer (10 mM Tris-HCl, pH 8.5)	2.5 ml
ChargeSwitch®-Pro PCR Clean-up Columns, inserted in Collection Tubes	10
ChargeSwitch®-Pro PCR Elution Tubes	10

Shipping and Storage

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

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.

Important Guidelines and Parameters

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.

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.

Buffers
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

- **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.

Centrifugation Protocol

Follow the steps below to purify PCR fragments using a microcentrifuge. All steps are performed at room temperature. For a protocol using a vacuum manifold and pump, see the next page.

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.)

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.

Vacuum Protocol

Follow the steps below to purify PCR fragments using a vacuum manifold and pump (producing pressure of 13–15 in. Hg or –450 to –550 mbar). All steps are performed at room temperature. For a protocol using only a microcentrifuge, see the previous page.

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 until the liquid has passed through the column. Then proceed to **Washing the Column**.

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

7. 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.)
8. Centrifuge the column/tube at 10,000 × *g* for 30–60 seconds. **The flow-through contains the purified DNA.**
9. 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.

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
3. 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

Problem	Possible 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.

Additional Products

The table below lists additional products available from Invitrogen.

Product	Quantity	Catalog no.
ChargeSwitch®-Pro PCR Clean-up Kit	50 preps 250 preps	CS32050 CS32250
ChargeSwitch® PCR Clean-up 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	P7589
PureLink™ Quick Gel Extraction Kit	50 preps 250 preps	K2100-12 K2100-25

A large selection of ChargeSwitch® products is available from Invitrogen for plasmid and genomic DNA purification from various sources.

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.

Purchaser Notification

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