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PureLink™ PCR Purification Kit

USER GUIDE

For rapid, efficient purification of PCR products

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Revision F

ThermoFisher SCIENTIFIC

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: MAN0000446 F (English)

Revision	Date	Description
F	2 April 2025	• Updated to the current document template, with associated updates to the limited license information, warranty, trademarks, and logos. • The contents and storage table was updated to include additional instructions for storage of purification columns.
E	10 June 2024	The number of bottles and preparation instructions for the Wash Buffer (W1) provided in Cat. No. K310002 were updated.
D	26 May 2011	Baseline for this revision history.

The information in this guide is subject to change without notice.

Contents

1 Product information

Product description

The PureLink™ PCR Purification Kit is designed to efficiently remove primers, dNTPs, enzymes, and salts from PCR products in less than 15 minutes. The kit is based on the selective binding of dsDNA to silica-based membrane in the presence of chaotropic salts.

The kit is supplied with two different Binding Buffers that can be used to set the cutoff range of eliminated fragments.

- Use Binding Buffer (B2) to purifying double stranded PCR products of 100 bp–12 kb.
- Use Binding Buffer High-Cutoff (HC) (B3) to remove primer dimers or short spurious PCR products (<300 bp) without the need for tedious gel purification.

The purified PCR product is suitable for automated fluorescent DNA sequencing, restriction enzyme digestion, and cloning.

Features

- Efficiently remove primers, dNTPs, salts, and enzymes without the need to perform ethanol precipitation.
- Purify PCR products in less than 15 minutes.
- Choose between Binding Buffers for routinely purifying PCR products or selectively removing primer dimers (<300 bp) and short spurious PCR products.
- Obtain reliable performance of the purified PCR products in downstream applications.

Kit specifications

Parmeter	Specification
Starting Material	50–100 µL PCR product (50 ng–40 µg dsDNA)
Elution Volume	50 µL
Separation Range: • Binding Buffer (B2) • Binding Buffer HC (B3)	 • Separates 0.1–12 kb from 10–40 mer primers • Separates >600 bp from <300 bp and 10–40 mer primers
DNA Recovery	>80%
Primer Removal	>99%
Binding Capacity	40 µg dsDNA per column
Column Reservoir Capacity	800 µL
Elution Tube Capacity	1.7 mL
Centrifuge Compatibility	>10,000 × *g*

Contents and storage

All components of the PureLink™ PCR Purification Kit are shipped at room temperature. Upon receipt, store all components at room temperature.

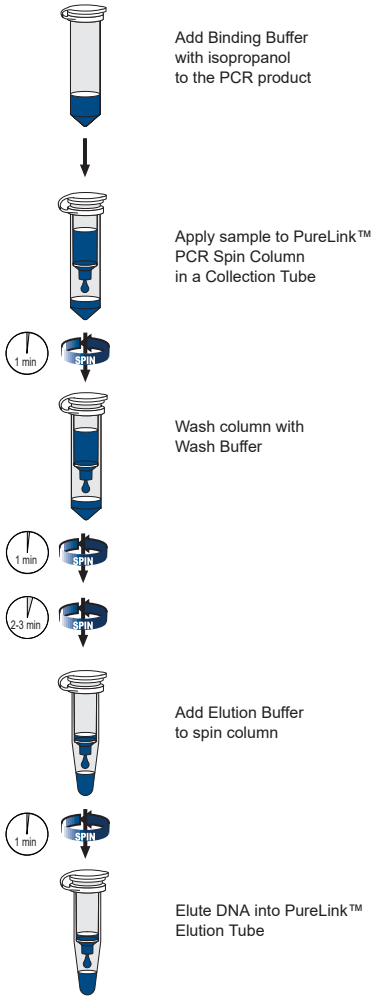
Note: For better long-term performance store PureLink™ PCR Spin Columns with Collection Tubes at 2°C to 8°C.

Component	Cat. no. K3100-01 (50 reactions)	Cat. no. K3100-02 (250 reactions)
Binding Buffer (B2)	15 mL	72 mL
Binding Buffer High-Cutoff (B3) [1]	23 mL	109 mL
Wash Buffer (W1)	16 mL	2 ×40 mL
Elution Buffer; 10 mM Tris-HCl, pH 8.5 (E1)	15 mL	15 mL
PureLink™ PCR Spin Columns with Collection Tubes	50	5 × 50
PureLink™ Elution Tubes (1.7 mL)	50	5 × 50

[1] Binding Buffer HC (B3) reduces the recovery of dsDNA fragments between 300–600 bp and prevents dsDNA fragments <300 bp from binding to PureLink™ Spin Columns.

PCR purification workflow

To purify PCR products using the PureLink™ PCR Purification Kit, mix PCR product with Binding Buffer to allow the dsDNA to bind to the silica-based membrane of the PureLink™ Spin Column. Add Wash Buffer and centrifuge to remove impurities, and elute purified DNA in low salt Elution Buffer or water.



2 Methods

Before first use of the kit

Before beginning, prepare Binding Buffer (B2) and Binding Buffer HC (B3) with isopropanol, and Wash Buffer (W1) with ethanol according to the following directions. After adding isopropanol or ethanol, store all buffers at room temperature.

Add ethanol to Wash Buffer (W1)

1. Add 96–100% ethanol to the bottle of Wash Buffer (W1).

Component	Cat. no. K3100-01 (16 mL Wash Buffer)	Cat. no. K3100-02 (80 mL Wash Buffer)
96–100% ethanol	64 mL	320 mL

2. Mark the checkbox on the Wash Buffer label to indicate that ethanol was added to the bottle.

Add isopropanol to Binding Buffer (B2)

1. Add 100% isopropanol to the bottle of Binding Buffer (B2).

Component	Cat. no. K3100-01 (15 mL Binding Buffer)	Cat. no. K3100-02 (72 mL Binding Buffer)
100% isopropanol	10 mL	48 mL

2. Mark the checkbox on the Binding Buffer label to indicate that isopropanol was added to the bottle.

Add isopropanol to Binding Buffer HC (B3)

1. Add 100% isopropanol to the bottle of Binding Buffer HC (B3).

Component	Cat. no. K3100-01 (23-mL Binding Buffer)	Cat. no. K3100-02 (109-mL Binding Buffer)
100% isopropanol	2.3 mL	11 mL

2. Mark the checkbox on the Binding Buffer label to indicate that isopropanol was added to the bottle.

General guidelines

- Maintain a PCR volume of 50–100 µL.
- Save a sample of the PCR products before purification to check the amplicon on a gel.
- Use a centrifuge at room temperature for all steps.
- Pipet the Elution Buffer (E1) in the center of the column and perform a 1 minute incubation.
- Always use sterile water with pH 7–8.5, if you are using water for elution.

CAUTION! The PureLink™ PCR Purification Kit buffers contain guanidine hydrochloride and isopropanol. Always wear a laboratory coat, disposable gloves, and eye protection when handling buffers.

Do not add bleach or acidic solutions directly to solutions containing guanidine hydrochloride or sample preparation waste because it forms reactive compounds and toxic gases when mixed with bleach or acids.

Required materials not supplied

Components required but not supplied:

- 100% isopropanol
- 96–100% ethanol
- Microcentrifuge capable of achieving >10,000 × *g*
- DNase-free pipettes and tips
- (*Optional*) Sterile, distilled water (pH>7.0)

PCR purification protocol

The procedure is used to purify up to 15 µg dsDNA using a centrifuge in a total time of 10–12 minutes.

Bind DNA

1. Add 4 volumes of Binding Buffer (B2) with isopropanol or Binding Buffer HC (B3) with isopropanol (see page 7) to 1 volume of the PCR product (50–100 µL). Mix well.
2. Remove a PureLink™ Spin Column in a Collection Tube from the package.
3. Add the diluted PCR sample to the PureLink™ spin column.
4. Centrifuge the column at room temperature at 10,000 × *g* for 1 minute.
5. Discard the flow through and place the spin column into the collection tube.

Wash DNA

1. Add 650 µL of Wash Buffer with ethanol (see page 7) to the spin column.
2. Centrifuge the column at room temperature at 10,000 × *g* for 1 minute. Discard the flow through from the collection tube and place the column back into the tube.
3. Centrifuge the column at maximum speed at room temperature for 2–3 minutes to remove any residual Wash Buffer. Discard the collection tube.

Elute DNA

1. Place the spin column in a clean 1.7-mL PureLink™ Elution Tube (supplied with the kit).
2. Add 50 µL of Elution Buffer (E1) or sterile, distilled water (pH >7.0) to the center of the column.
3. Incubate the column at room temperature for 1 minute.
4. Centrifuge the column at maximum speed for 2 minutes.
5. *The elution tube contains the purified PCR product.* Remove and discard the column. The recovered elution volume is ~48 µL.
6. Store the purified PCR product at –20°C or use the PCR product for the desired downstream application.

Analyze DNA yield and efficiency of primer removal

After purifying DNA with the PureLink™ PCR Purification Kit, estimate the yield of purified dsDNA and efficiency of primer removal.

Determine DNA yield

Estimate the yield of purified dsDNA with Qubit™ DNA Assay Kits, or by agarose gel electrophoresis.

Determine DNA yield with Qubit™ DNA Assay Kits

Measure DNA concentration using Qubit™ DNA Assay Kits or UV absorbance at 260 nm.

The Qubit™ DNA Assay Kits (see "Accessory products" on page 13 for ordering information) provide a rapid, sensitive, and specific method for measuring dsDNA concentration with minimal interference from RNA, protein, ssDNA (primers), or other common contaminants that affect UV absorbance.

The kit contains a state-of-the-art quantitation reagent, pre-diluted standards for standard curve, and a pre-made buffer. For optimal results, perform the quantitation using a Qubit™ 4 Fluorometer (see "Accessory products" on page 13). You can also use standard fluorescent microplate readers. Follow manufacturer's recommendations to perform the assay.

Determine DNA yield by agarose gel electrophoresis

Perform agarose gel electrophoresis with the purified PCR product and known quantities of DNA fragments of the same size. Compare the band intensities of the purified PCR product with the standard DNA fragments. The band intensity of the known standard that approximates the band intensity of the PCR product provides an estimate of the DNA yield.

Estimate primer removal

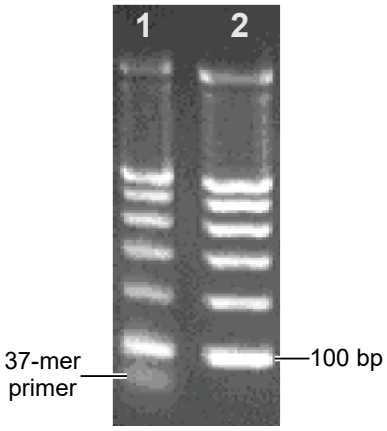
Estimate the efficiency of primer removal with agarose gel electrophoresis as described in the examples shown in "Expected results" on page 11.

The WAVE™ System is an ideal method to estimate the efficiency of primer removal. The WAVE™ System is an automated DHPLC (denatured high-performance liquid chromatography) system.

Expected results

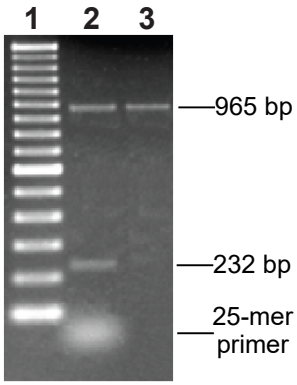
Some examples of sample purification are described in the following section.

A 100 bp DNA Ladder (see "Accessory products" on page 13) sample spiked with an excess of a 37-mer primer was purified according to the protocol using Binding Buffer (B2). The spiked ladder and purified sample were analyzed using agarose gel electrophoresis, and shows removal of the primer.



- Lane 1: Sample before purification
- Lane 2: Sample after purification

PCR product was purified according to the protocol using Binding Buffer HC (B3). The PCR product was analyzed by agarose gel electrophoresis and shows efficient removal of <300 bp fragments using Binding Buffer HC (B3). The 232-bp PCR fragment and primers are removed after purification, leaving the 965-bp PCR product.



- Lane 1: DNA ladder
- Lane 2: PCR product before purification
- Lane 3: PCR product after purification

A Troubleshooting

Observation	Cause	Solution
Low DNA yield	PCR conditions are not optimized	Check the amplicon on the gel to verify the PCR product prior to purification.
	Incorrect binding conditions	For efficient DNA binding, always **mix** 1 volume of PCR (50–100 µL) with 4 volumes of Binding Buffer. Be sure to add 100% isopropanol to the Binding Buffer as described in "Bind DNA" on page 9.
	Ethanol not added to Wash Buffer	Add 96–100% ethanol to Wash Buffer as described in "Before first use of the kit" on page 7.
	Incorrect elution conditions	Add Elution Buffer to the center of the column. Incubate the column for 1 minute with the Elution Buffer before centrifugation.
Primer dimers present	Incorrect Binding Buffer used	To efficiently remove primer dimers or short spurious PCR products (<300 bp), use Binding Buffer HC (B3). Binding Buffer HC (B3) is specifically designed to remove <300 bp DNA fragments, eliminating the need for gel purification.
Downstream enzymatic reactions are inhibited	Ethanol present in purified DNA	Traces of ethanol from the Wash Buffer can inhibit downstream enzymatic reactions. To remove Wash Buffer, discard Wash Buffer flow through from the collection tube. Place the spin column into the collection tube and centrifuge the spin column at maximum speed for 2–3 minutes to completely dry the column.

B

Related products

Accessory products

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Product	Quantity	Cat. no.
PureLink™ *Pro* 96 PCR Purification Kit	4 × 96 reactions	K3100-96A
Platinum™ *Taq* DNA Polymerase High Fidelity	100 reactions	11304-011
Platinum™ *Taq* DNA Polymerase	100 reactions	10966-018
UltraPure™ DNase/RNase-free Distilled Water	500 mL	10977-015
Qubit™ dsDNA Assay Kit, High Sensitivity	500 assays	Q32854
Qubit™ dsDNA Assay Kit, Broad-Range	500 assays	Q32853
Qubit™ 2.0 Fluorometer	1 each	Q32857
PureLink™ 96 Receiver Plate	50	12193-025
100 bp DNA Ladder	50 µg	15628-019

C Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, visit **thermofisher.com/support**.

Documentation and support

Customer and technical support

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- Product documentation
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

  Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and its affiliates warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have questions, contact Life Technologies at **www.thermofisher.com/support**.