USER GUIDE

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PureLink® PCR Purification Kit

For rapid, efficient purification of PCR products

Catalog numbers K3100-01 and K3100-02

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Contents

Kit Contents and Storage

Shipping and storage

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

Kit contents

The components included in the PureLink® PCR Purification Kit are listed below.

Sufficient reagents are provided in the kit to perform 50 (Cat. no. K3100-01) or 250 (Cat. no. K3100-02) reactions.

Component	Cat. no. K3100-01	Cat. no. K3100-02
Binding Buffer (B2)	15 mL	72 mL
Binding Buffer High-Cutoff (B3)	23 mL	109 mL
Wash Buffer (W1)	16 mL	80 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

Intended use

For research use only. Not intended for any animal or human therapeutic or diagnostic use.

Description of the System

About the Kit

System overview

Use the PureLink® PCR Purification Kit to efficiently remove primers, dNTPs, enzymes, and salts from PCR products in less than 15 minutes. Use the kit with Binding Buffer High-Cutoff (B3) to remove primer dimers or short spurious PCR products (see **PureLink® Binding Buffers** below).

The PureLink® PCR Purification Kit is based on the selective binding of dsDNA to silica-based membrane in the presence of chaotropic salts.

When you use the kit, you will mix a PCR product with Binding Buffer to adjust conditions for subsequent dsDNA binding to the PureLink® Spin Column. The dsDNA binds to the silica-based membrane in the column. Remove impurities by thorough washing with Wash Buffer. To purify the DNA, elute the dsDNA in low salt Elution Buffer or water.

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

PureLink® Binding Buffers

The PureLink® PCR Purification Kits are supplied with two proprietary buffers:

- Binding Buffer (B2): For purifying 100 bp–12 kb dsDNA PCR fragments.
- Binding Buffer High-Cutoff (HC) (B3): For removing primer dimers or short spurious PCR products (<300 bp), eliminating the need for tedious gel purification.

Note: Binding Buffer HC (B3) reduces the recovery of dsDNA fragments between 300–600 bp and prevents dsDNA fragments <300 bp from binding to the PureLink® Spin Column.

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About the Kit, Continued

Advantages of the PureLink® PCR Purification Kit

- 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.

PureLink® PCR Spin Column specifications

Binding Capacity:	40 µg dsDNA
Column Reservoir Capacity:	800 µL
Elution Tube Capacity:	1.7 mL
Centrifuge Compatibility:	Capable of centrifuging >10,000 × *g*

System specifications

Starting Material:	50–100 µL PCR product (50 ng–40 µg dsDNA)
Elution Volume:	50 µL
Separation Range: Binding Buffer (B2)	0.1–12 kb from 10–40 mer primers
Separation Range: Binding Buffer HC (B3)	>600 bp from <300 bp PCR fragments and 10–40 mer primers
DNA Recovery:	>80%
Primer Removal:	>99%

Experimental Overview

Purifying PCR products workflow

The flow chart for purifying PCR products using the PureLink® PCR Purification Kit is shown below.

Add appropriate Binding Buffer with isopropanol to the PCR products

Apply sample to PureLink® PCR Spin Column in a Collection Tube

Wash column with Wash Buffer

Elute DNA into PureLink® Elution Tube

Methods

Procedure for Purifying PCR Products

Introduction

The procedure is designed for purifying up to **40 µg dsDNA** using a centrifuge in a total time of **10–12 minutes**.

Required materials

Components required but not supplied:

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

Components supplied with the kit:

- Binding Buffer (B2 or B3)
- Wash Buffer (W1)
- Elution Buffer (E1)
- PureLink® PCR Spin Column and Collection Tubes
- PureLink® Elution Tubes

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.

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Procedure for Purifying PCR Products, Continued

Follow the recommendations below to obtain the best results:

- Maintain a PCR volume of 50–100 µL.
- Save an aliquot of PCR products before purification to verify and check the amplicon on the 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.

Before starting

Add isopropanol to the Binding Buffers and ethanol to the Wash Buffer according to the following table. After adding isopropanol or ethanol, store all buffers at room temperature.

Buffer	Cat. no. K3100-01	Cat. no. K3100-02
Binding Buffer (B2)	10 mL 100% Isopropanol	48 mL 100% Isopropanol
Binding Buffer HC (B3)	2.3 mL 100% Isopropanol	11 mL 100% Isopropanol
Wash Buffer (W1)	64 mL 96–100% Ethanol	320 mL 96–100% Ethanol

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Procedure for Purifying PCR Products, Continued

Binding DNA

1. Add 4 volumes of PureLink® Binding Buffer (B2) with isopropanol (see page 5) or Binding Buffer HC (B3) with isopropanol (see page 5) 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 sample with the appropriate Binding Buffer (from step 1 of this procedure) 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.

   Proceed to **Washing DNA**.

Washing DNA

1. Add 650 µL of Wash Buffer with ethanol (see page 5) to the 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 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.

   Proceed to **Eluting DNA**.

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Procedure for Purifying PCR Products, Continued

Eluting 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 (10 mM Tris-HCl, pH 8.5) 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.

Examples of efficient primer removal using the different Binding Buffers are described on page 9.

Analyzing DNA Yield and Primer Removal

DNA yield

After purifying DNA with the PureLink® PCR Purification Kit, estimate the yield of purified dsDNA by agarose gel electrophoresis or with Qubit® DNA Assay Kits.

Qubit® DNA Assay Kits

The Qubit® DNA Assay Kits (see page 11 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 kits contain a state-of-the-art quantitation reagent, pre-diluted standards for standard curve, and a pre-made buffer. The assay is designed for reading in standard fluorescent readers/fluorometer or Qubit® 2.0 Fluorometer.

Agarose Gel Electrophoresis

To estimate DNA yield, 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.

Primer removal

Estimate the efficiency of primer removal with agarose gel electrophoresis as described in the examples shown on page 9.

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.

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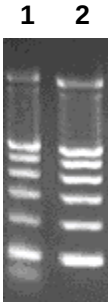
Analyzing DNA Yield and Primer Removal, Continued

Example with Binding Buffer

An example of efficient primer removal using Binding Buffer is shown below.

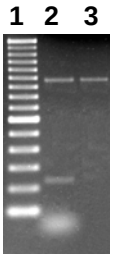
A mixture of 100 bp DNA Ladder (see page 11) with an excess of a 37-mer primer was purified using Binding Buffer with this PureLink® PCR Purification Kit as described in the manual. The mixture was analyzed using agarose gel electrophoresis before purification (Lane 1) and after purification (Lane 2).



Example with Binding Buffer HC (B3)

An example of efficient removal of <300 bp fragments using Binding Buffer HC (B3) is shown below.

PCR product was purified using Binding Buffer HC (B3) with PureLink® PCR Purification Kit as described in this manual. The PCR product was analyzed by agarose gel electrophoresis and shows efficient purification of a 965-bp PCR product from a 232-bp PCR fragment and primers. The figure below shows a DNA Ladder (Lane 1), the PCR product before purification (Lane 2), and the PCR product after purification (Lane 3).



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 on page 6.
	Ethanol not added to Wash Buffer	Add 96–100% ethanol to Wash Buffer as described on page 5.
	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.

Appendix

Accessory Products

Introduction

The following products may be used with the PureLink® PCR Purification Kit. For details, visit www.invitrogen.com or contact **Technical Support** (see page 12).

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

Technical Support

Obtaining support

For the latest services and support information for all locations, go to www.invitrogen.com.

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support (techsupport@invitrogen.com)
- Search for user documents, Safety Data Sheets (SDSs), vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

SDS

Safety Data Sheets (SDSs) are available at www.invitrogen.com/sds.

Certificate of analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to www.invitrogen.com/support and search for the Certificate of Analysis by product lot number, which is printed on the box.

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Technical Support, Continued

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