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PureLink® PCR Purification Kit

For rapid, efficient purification of PCR products

Catalog nos. K3100-01, K3100-02

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Manual part no. 25-0715

MAN0000446

User Manual

Contents

Experienced Users Procedure

Note

This quick reference sheet is included for experienced users of the PureLink® PCR Purification Kit. If you are a first time user, follow the detailed protocol provided in this manual.

Purification Procedure

The purification procedure is designed for purifying up to 40 µg dsDNA in a total time of 10–12 minutes using a microcentrifuge capable of centrifuging >10,000 × *g*.

1. Add 4 volumes of the appropriate PureLink® Binding Buffer with isopropanol (page 7) to 1 volume of PCR (50–100 µL). Mix well.
2. **Add** sample from Step 1 to a PureLink® Spin Column in a collection tube.
3. Centrifuge the column at 10,000 × *g* for 1 minute. dsDNA is bound to the column. Discard the flow through.
4. **Wash** the column with 650 µL of Wash Buffer with ethanol (page 6).
5. Centrifuge the column at 10,000 × *g* for 1 minute. Discard the flow through.
6. Centrifuge the column at maximum speed for 2–3 minutes to remove any residual wash buffer.
7. Place the spin column in a clean 1.7-mL elution tube supplied with the kit.
8. **Elute** with 50 µL of Elution Buffer (add buffer to the center of the column).
9. Incubate the column at room temperature for 1 minute.
10. Centrifuge the column at maximum speed for 2 minutes.
11. The elution tube contains your purified PCR product. Discard the column. Store the purified PCR product at –20°C or use PCR product for the desired downstream application.

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	K3100-01	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.

Introduction

Product Overview

Description of the System

The PureLink® PCR Purification Kit is designed for rapid and efficient purification of PCR products.

The kit is designed to efficiently remove primers, dNTPs, enzymes, and salts from PCR products in less than 15 minutes. Using the kit with Binding Buffer HC (High-Cutoff) efficiently removes primer dimers or short spurious PCR products (see **PureLink®. Binding Buffers**, below).

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

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

The PCR product is mixed 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 and impurities are removed by thorough washing with Wash Buffer. The dsDNA is then eluted in low salt Elution Buffer or water.

PureLink® Binding Buffers

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

- Binding Buffer: For routine purifications of 100 bp to 12 kb dsDNA PCR fragments
- Binding Buffer HC (High-Cutoff): For removal of primer dimers or short failed PCR products (<300 bp), eliminating the need for tedious gel purification.

Note: Using Binding Buffer HC reduces the recovery of dsDNA fragments between 300–600 bp, while dsDNA fragments <300 bp do not bind to the PureLink® Spin Column.

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Product Overview, Continued

Advantages

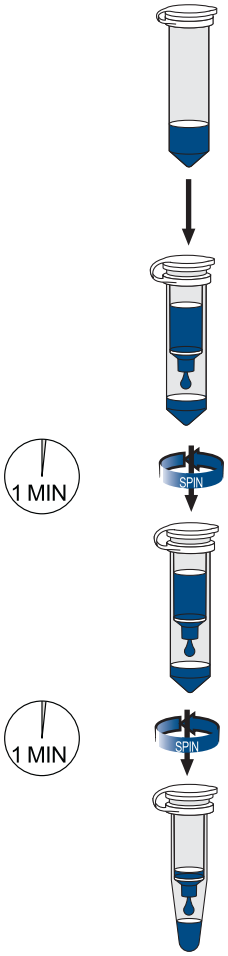
The advantages of using PureLink® PCR Purification Kit are:

- Efficient removal of primers, dNTPs, salts, and enzymes without the need to perform ethanol precipitation
- Designed to purify PCR products in less than 15 minutes
- Choice of Binding Buffers for routine purification or selective removal of primer dimers (<300 bp) and short spurious PCR products
- Reliable performance of the purified PCR products in downstream applications

Experimental Overview

Scheme

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

Product Specifications

PureLink® PCR Spin Column	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)	0.1–12 kb from 10–40 mer primers
	Separation Range: (Binding Buffer HC)	>600 bp from <300 bp PCR fragments and 10–40 mer primers
	DNA Recovery:	>80%
	Primer Removal:	>99%

Methods

Purification Procedure

Introduction

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

Materials Needed

- 100% isopropanol
- 96–100% ethanol
- Binding Buffer (supplied with the kit)
- Wash Buffer (supplied with the kit)
- Elution Buffer (supplied with the kit)
- PureLink® PCR Spin Column and Collection Tubes (supplied with the kit)
- PureLink® Elution Tubes (supplied with the kit)
- Sterile, distilled water (pH>7.0)
- Microcentrifuge capable of achieving >10,000 × *g*

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 as it forms reactive compounds and toxic gases when mixed with bleach or acids.

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Purification Procedure, Continued

Follow the recommendations below to obtain the best results:

- Recommended PCR volume is 50–100 µL
- Save an aliquot of PCR products before purification to verify and check amplicon on the gel
- Perform all centrifugation steps at room temperature
- Pipet the Elution Buffer 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 10 mL 100% isopropanol to 15 mL Binding Buffer included with Cat. no. K3100-01.

  Add 48 mL 100% isopropanol to 72 mL Binding Buffer included with Cat. no. K3100-02

  Store the Binding Buffer with isopropanol at room temperature.
- Add 2.3 mL isopropanol to 23 mL Binding Buffer HC included with Cat. no. K3100-01.

  Add 11 mL 100% isopropanol to 109 mL Binding Buffer HC included with Cat. no. K3100-02

  Store the Binding Buffer HC with isopropanol at room temperature.
- Add 64 mL 96–100% ethanol to 16 mL Wash Buffer included with Cat. no. K3100-01.

  Add 320 mL 96–100% ethanol to 80 mL Wash Buffer included with Cat. no. K3100-02

  Store the Wash Buffer with ethanol at room temperature.

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Purification Procedure, Continued

Binding DNA

1. Add 4 volumes of PureLink® Binding Buffer with isopropanol (see page 6) or Binding Buffer HC with isopropanol (see page 6) 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 sample with appropriate Binding Buffer from Step 1 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.
6. Proceed to **Washing DNA**, below.

Washing DNA

1. Add 650 µL of Wash Buffer with ethanol (page 6) 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.
4. Proceed to Eluting DNA, next page.

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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 your 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 PCR product for the desired downstream application.

Examples of efficient primer removal using the different Binding Buffers are described on page 10.

Analyzing DNA Yield and Primer Removal

DNA Yield

After purification with PureLink® PCR Purification Kit, the yield of purified dsDNA can be estimated by agarose gel electrophoresis or Quant-iT™ DNA Assay Kits.

Agarose Gel Electrophoresis

To estimate the yield, perform agarose gel electrophoresis of the purified PCR product and known quantities of DNA fragment of the same size. Compare the band intensity of the purified PCR product with the standard DNA fragments.

Quant-iT™ DNA Assay Kits

The Quant-iT™ DNA Assay Kits (see page 12 for ordering information) provide a rapid, sensitive, and specific method for dsDNA quantitation 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 ready-to-use buffer. The assay is performed in a microtiter plate format and is designed for reading in standard fluorescent microplate readers. Follow manufacturer's recommendations to perform the assay.

Primer Removal

The efficiency of primer removal can be estimated by agarose gel electrophoresis as described in the examples shown on the next page.

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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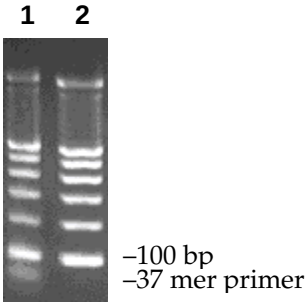
Analyzing DNA Yield and Primer Removal, Continued

Example with Binding Buffer

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

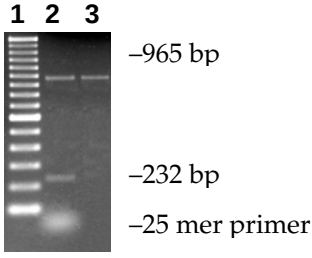
A mixture of 100 bp DNA Ladder (see page 12) with an excess of a 37 mer primer was purified using Binding Buffer with the PureLink® PCR Purification Kit as described in the manual. The mixture was analyzed using agarose gel electrophoresis before purification (lane 1) and after purification (lane 2).



Example with Binding Buffer HC

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

PCR product was purified using Binding Buffer HC with PureLink® PCR Purification Kit as described in the 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 not optimized	Check amplicon on 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 7.
	Ethanol not added to Wash Buffer	Be sure to add 96–100% ethanol to Wash Buffer as described on page 6.
	Incorrect elution conditions	Add elution buffer to the center of the column and perform incubation for 1 minute with elution buffer before centrifugation.
Presence of primer dimers	Incorrect Binding Buffer used	For efficient removal of primer dimers or short failed PCR products (<300 bp), use Binding Buffer HC. This buffer is specifically designed to remove <300 bp DNA fragments eliminating the need for gel purification.
Inhibition of downstream enzymatic reactions	Presence of ethanol 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 13).

Item	Amount	Cat. no.
PureLink® 96 PCR Purification Kit	4 × 96 reactions	K3100-96
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
Quant-iT™ DNA Assay Kit, High Sensitivity	1000 assays	Q33120
Quant-iT™ DNA Assay Kit, Broad-Range	1000 assays	Q33130
PureLink™ 96 Receiver Plate	50	12193-025
100 bp DNA Ladder	50 µg	15628-019

Technical Support

Web Resources

Visit the Invitrogen website at **www.invitrogen.com** for:

- Technical resources, including manuals, vector maps and sequences, application notes, SDSs, FAQs, formulations, citations, handbooks, etc.
- Complete technical support contact information
- Access to the Invitrogen Online Catalog
- Additional product information and special offers

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SDS Information

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

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Technical Support, Continued

Limited Warranty

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Notes

Notes

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