invitrogen™

PureLink™ PCR Micro Kit

For rapidly purifying and concentrating DNA from PCR reactions in low elution volumes

Catalog nos. K310050, K310250

Rev. Date: 29 July 2009
Part no. 100003661

MAN0000408

IMPORTANT!

New pre-spin step added to protocol

User Manual

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Experienced Users Procedure

Introductions

This quick reference page is provided for experienced users of the PureLink™ PCR Micro Kit. If you are a first time user of this kit, refer to the details provided in this manual.

Purification Procedure

Before starting, prepare Wash Buffer (W1) with 100% ethanol and Binding Buffer (B2) with 100% isopropanol (page 5).

Binding, Washing and Elution of DNA

Follow the steps below to bind, wash and elute the DNA from your PCR product.

1. Before using the PureLink™ Micro Kit Column, centrifuge the column with collection tube for 1 minute at 10,000 × g.
2. Add 4 volumes of Binding Buffer (B2) with isopropanol to 1 volume of your PCR product. (e.g. add 200 µl Binding Buffer (B2) with isopropanol to 50 µl PCR product).
3. Vortex or invert tube repeatedly to mix thoroughly, then transfer the entire PCR product with Binding Buffer to a PureLink™ Micro Kit Column with a Collection Tube.
4. Centrifuge at 10,000 × g for 1 minute at room temperature.
5. Add 650 µl Wash Buffer (W1) with ethanol to your sample in the PureLink™ Micro Kit Column.
6. Centrifuge at 10,000 × g for 1 minute at room temperature. Discard the flow–through and reinsert the PureLink™ Micro Kit Column into the Collection Tube.
7. Centrifuge at 14,000 × g for 1 minute to dry the silica membrane and remove any residual Wash Buffer with ethanol. Discard the flow–through **and** the Collection Tube. Reinsert the PureLink™ Micro Kit Column into an Elution Tube.
8. Add 10 µl Elution Buffer (E1, 10 mM Tris–HCl, pH 8.5) to the center of the PureLink™ Micro Kit Column.
9. Incubate for 1 minute at room temperature.
10. Centrifuge at 14,000 × g for 1 minute to collect the purified DNA into the Elution Tube. Remove and **discard** the PureLink™ Micro Kit Column. The Elution Tube now contains your purified DNA.

The recovered elution volume is ~9–10 µl. For long–term storage, store the purified DNA at –20°C.

Kit Contents and Storage

Shipping and Storage

All contents of the PureLink™ PCR Micro Kits are shipped at room temperature.

Upon receipt, store all contents at room temperature. Kit contents are guaranteed stable for six months when properly stored.

Kit Contents

The components included in the PureLink™ PCR Micro Kits are described below.

Sufficient reagents are provided to perform 50 preps for catalog number K310050 and 250 preps for catalog number K310250.

Component	Quantity	
	K310050	**K310250**
Binding Buffer (B2)	15 ml	72 ml
Wash Buffer (W1)	8 ml	40 ml
Elution Buffer (E5) (10 mM Tris-HCl, pH 8.5)	15 ml	15 ml
PureLink™ Micro Kit Columns (with Collection Tubes)	50 each	5 × 50 each
PureLink™ Elution Tubes	50 each	5 × 50 each

Accessory Products

Additional Products

The following products are also available from Invitrogen.

For more details, visit our web site at www.invitrogen.com or contact **Technical Support** (page 10).

Product	Quantity	Catalog No.
PureLink™ PCR Micro Kit	10 prep	K310010
PureLink™ PCR Purification Kit	50 preps 250 preps	K3100–01 K3100–02
PureLink™ 96 PCR Purification Kit	4 × 96 reactions	K3100-96
PureLink™ Quick Gel Extraction Kit	50 preps 250 preps	K2100-12 K2100-25
UltraPure™ DNase/RNase–Free Distilled Water	500 ml	10977–015
Platinum® PCR SuperMix High Fidelity	100 reactions	12532–016
Platinum® *Taq* DNA Polymerase High Fidelity	100 reactions	11304–011
Platinum® *Taq* DNA Polymerase	100 reactions	10966–018
Quant–iT™ DNA Assay Kit, High Sensitivity	1,000 assays	Q33140
Quant–iT™ DNA Assay Kit, Broad–Range	1,000 assays	Q33130
100 bp DNA Ladder	50 µg	15628–019
Low DNA Mass™ Ladder	50 applications	10068–013

E-Gel® Agarose Gels and DNA Ladders

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 for your convenience.

A large variety of DNA ladders is available from Invitrogen for sizing DNA.

For details on these products, visit our website at www.invitrogen.com or contact **Technical Support** (page 10).

Introduction

Overview

Introduction

The PureLink™ PCR Micro Kit is designed for rapid and efficient purification of DNA from PCR products ranging in size from 125 bp–12.3 kb. This kit allows you to isolate and purify high concentrations of DNA from PCR products in low elution volumes (10 µl). Using the PureLink™ PCR Micro Kit, >90% of dsDNA/ssDNA/primer dimmers less than 50 bp, as well as dNTPs, enzymes, and salts are removed from your PCR products in approximately 6 minutes.

System Overview

This manual provides a protocol for purifying high concentrations of DNA from PCR products in low elution volumes.

Binding Buffer (B2) containing isopropanol is mixed with your PCR product in a 4:1 ratio. This PCR product/Binding Buffer mixture is added to the PureLink™ Micro Kit Column with a Collection Tube and is then centrifuged, allowing the DNA to bind to the silica membrane of the column. Impurities are subsequently removed from the silica membrane by the addition of Wash Buffer (W1) containing ethanol. The purified DNA is then eluted into the Elution Tube using a low salt Elution Buffer (E1). The purified DNA is suitable for use in a wide variety of downstream applications (next page).

Binding Buffer

The PureLink™ PCR Micro Kits contains Binding Buffer (B2); a proprietary blend allowing for routine purifications of 125 bp–12.3 kb dsDNA PCR fragments.

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Advantages

The advantages of using the PureLink™ PCR Micro Kit are:

- High DNA concentrations in low elution volumes
- Efficient removal of primers, dNTPs, salts, and enzymes without the need to perform ethanol precipitation
- Efficient removal of unincorporated dye–labeled nucleotides from cDNA labeling reactions
- Minimal variation in elution volume recovery
- Purifies PCR products in ~6 minutes
- Excellent performance of the purified PCR products in downstream applications

Downstream Applications

The purified PCR product is suitable for any downstream application, including:

- DNA sequencing
- Cloning
- Restriction enzyme digestion
- PCR reactions
- Labeling

PureLink™ Micro Kit Column

Binding Capacity:	5 µg dsDNA
Column Reservoir Capacity:	800 µl
Collection Tube Capacity:	2.0 ml
Elution Tube Capacity:	1.7 ml
Centrifuge Compatibility:	Capable of centrifuging >14,000 × g

Kit Specifications

Starting Material:	50–100 µl PCR product
Elution Volume:	10 µl
Separation Range:	0.1–12 kb from 10–40 mer primers
DNA Recovery:	up to 95%
Primer Removal:	>95%
Processing time	~6 minutes

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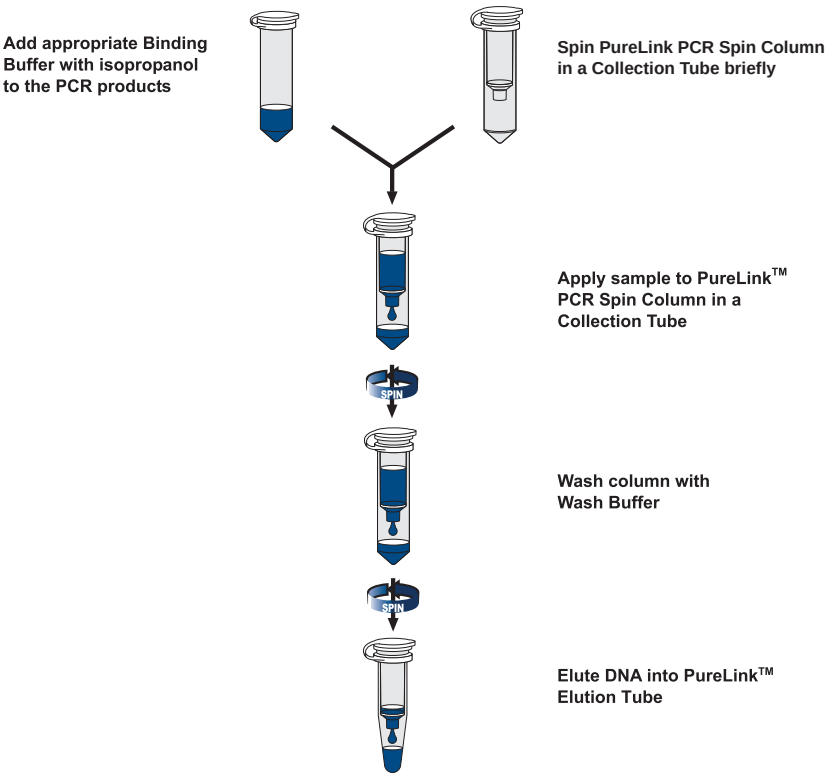
Workflow

The flow chart below illustrates the steps for purifying your PCR products using the PureLink™ PCR Micro Kit.



Methods

Purification Procedure

Introduction

This purification procedure is designed to purify up to 5 µg dsDNA using a centrifuge in a total time of ~6 minutes.

Materials Needed

You will need the following items:

- 100% isopropanol
- 100% ethanol
- Microcentrifuge capable of centrifuging >14,000 x g

Contents supplied with the kit:

- Binding Buffer (B2)
- Wash Buffer (W1)
- Elution Buffer (E1)
- PureLink™ Micro Kit Columns (with Collection Tube)
- PureLink™ Elution Tubes

- The Binding Buffer (B2) contains guanidine hydrochloride. This chemical is harmful when in contact with the skin, or when it is inhaled or ingested.
- **Do not** add bleach or acidic solutions directly to solutions or sample preparation waste that contains guanidinium hydrochloride, as reactive compounds and toxic gases are formed.
- The Wash Buffer (W1) contains ethanol and the Binding Buffer (B2) contains isopropanol. Solutions containing ethanol or isopropanol are considered flammable. Use appropriate precautions when using these chemicals.

For your protection, always wear a laboratory coat, gloves and safety glasses when handling these chemicals. Dispose of the buffers and chemicals in appropriate waste containers.

Important

Prior to using the PureLink™ Micro Kit Column, spin the column in the collection tube briefly at 10,000 × g. to ensure that the silica membrane is settled at the bottom of the column.

Preparing Binding Buffer with Isopropanol

Before beginning, prepare the Binding Buffer (B2) with 100% isopropanol as follows:

For K310050 (50 preps):

1. Add 10 ml 100% isopropanol to the Binding Buffer.
2. Check the box on the Binding Buffer label to indicate that isopropanol was added.
3. Store the Binding Buffer with isopropanol at room temperature.

For K310250 (250 preps):

1. Add 48 ml 100% isopropanol to the Binding Buffer.
2. Check the box on the Binding Buffer label to indicate that isopropanol was added.
3. Store the Binding Buffer with isopropanol at room temperature.

Preparing Wash Buffer with Ethanol

Before beginning, prepare the Wash Buffer (W1) with 100% ethanol as follows:

For K310050 (50 preps):

1. Add 32 ml 100% ethanol to the Wash Buffer.
2. Check the box on the Wash Buffer label to indicate that ethanol was added.
3. Store the Wash Buffer with ethanol at room temperature.

For K310250 (250 preps):

1. Add 160 ml 100% ethanol to the Wash Buffer.
2. Check the box on the Wash Buffer label to indicate that ethanol was added.
3. Store the Wash Buffer with ethanol at room temperature.

Follow the recommendations below to obtain the best results:

- Use the recommended PCR volume of 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.

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Binding Washing and Eluting DNA

Follow the steps below to bind, wash and elute the DNA from your PCR product.

1. Before using the PureLink™ Micro Kit Column, centrifuge the column with collection tube for 1 minute at 10,000 × g.
2. Add 4 volumes of Binding Buffer (B2) with isopropanol to 1 volume of your PCR product. (e.g. add 200 µl Binding Buffer (B2) with isopropanol to 50 µl PCR product).
3. Vortex or invert tube repeatedly to mix thoroughly, then transfer the entire PCR product with Binding Buffer to a PureLink™ Micro Kit Column with a Collection Tube.
4. Centrifuge at 10,000 × g for 1 minute at room temperature.
5. Add 650 µl Wash Buffer (W1) with ethanol to your sample in the PureLink™ Micro Kit Column.
6. Centrifuge at 10,000 × g for 1 minute at room temperature. Discard the flow–through and reinsert the PureLink™ Micro Kit Column into the Collection Tube.
7. Centrifuge at 14,000 × g for 1 minute to dry the silica membrane and remove any residual Wash Buffer with ethanol. Discard the flow–through **and** the Collection Tube. Reinsert the PureLink™ Micro Kit Column into an Elution Tube.
8. Add 10 µl Elution Buffer (E1, 10 mM Tris–HCl, pH 8.5) to the center of the PureLink™ Micro Kit Column.
9. Incubate for 1 minute at room temperature.
10. Centrifuge at 14,000 × g for 1 minute to collect the purified DNA into the Elution Tube. Remove and **discard** the PureLink™ Micro Kit Column. The Elution Tube now contains your purified DNA.

The recovered elution volume is ~9–10 µl. For long–term storage, store the purified DNA at –20°C. For immediate use, proceed to **Analyzing DNA Yield and Quality** (next page) or proceed to the downstream application of your choice.

Analyzing DNA Yield and Quality

DNA Yield

After product purification using the PureLink™ PCR Micro Kit, the yield of purified DNA can be estimated by agarose gel electrophoresis or Quant-iT™ DNA Assay Kits (see page 6).

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 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.

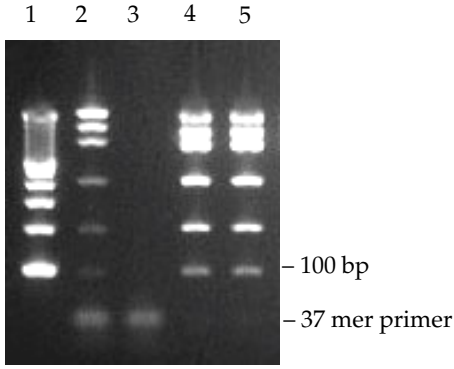
The WAVE® System is an ideal method to estimate the efficiency of primer removal. The WAVE® System is an automated DHPLC (denatured high-performance liquid chromatography) system.

Expected Results

An example of efficient primer removal using the PureLink™ PCR Micro Kit is shown below.

Five micrograms (5 µg) of Low DNA Mass™ Ladder (page vii) was mixed with an excess of a 37 mer primer and Binding Buffer with 100% isopropanol. The mixture was purified using with the PureLink™ PCR Micro Kit as described in the manual.

The Binding Buffer/DNA ladder/primer mixture was analyzed prior to purification (Lane 2) and after purification (Lanes 4–5) using agarose gel electrophoresis. Lane 1 is a 100 bp DNA Ladder (page vii), and Lane 3 is the 37 mer primer only.



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Troubleshooting

Problem	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 reaction with 4 volumes of Binding Buffer (B2) containing isopropanol. Be sure to add 100% isopropanol to the Binding Buffer as described on page 5.
	Ethanol not added to Wash Buffer	Be sure to add 100% ethanol to the Wash Buffer (W1, page 5) **before** use.
	Membrane not seated properly in column	Perform pre-spin of PureLink™ Micro Kit Column before adding sample.
	Incorrect elution conditions	Be sure to add Elution Buffer (E1) to the center of the column and perform incubation for 1 minute with Elution Buffer before centrifugation.
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 the Wash Buffer, discard the Wash Buffer flow through and centrifuge the spin column again at 14,000 × g for 1 minute to completely dry the column.

Technical Support

Web Resources

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.**

Certificate of Analysis

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