Trouble-shooting

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 **before** use.
	Ethanol not added to Wash Buffer	Be sure to add 100% ethanol to the Wash Buffer (W1) **before** use.
	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.

Accessory Products

The following products are also available from Invitrogen. For more details, visit our web site at www.invitrogen.com or contact **Technical Support.**

Product	Quantity	Catalog No.
PureLink™ PCR Micro Kit	50 preps 250 preps	K310050 K310250
PureLink™ PCR Purification Kit	50 preps 250 preps	K3100–01 K3100–02
PureLink™ 96 PCR Purification Kit	4 × 96 reactions	K3100-96
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

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 are available from Invitrogen for sizing DNA.

For more details on these products, visit our website at www.invitrogen.com or contact **Technical Support**.

Certificate of Analysis

The Certificate of Analysis (CofA) provides detailed quality control information for each product. The CofA is available on our website at www.invitrogen.com/cofa, and is searchable by product lot number, which is printed on each box.

invitrogen™

PureLink™ PCR Micro Kit

Catalog no. K310010 **Quantity: 10 preps**

Store at room temperature

Description

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 with low elution volumes (10 µl). Using the PureLink™ PCR Micro Kit, >90% of dsDNA/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 document provides a protocol for purifying high concentrations of DNA from PCR products with low elution volumes.

Binding Buffer (B2) containing isopropanol is mixed with your PCR product in a 4:1 ratio. Your PCR product is then 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, and 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 such as restriction enzyme digestion, cloning and automated fluorescent DNA sequencing.

Contents and Storage

All contents of the PureLink™ PCR Micro Kit 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.

Component	Quantity
Binding Buffer (B2)	6 ml
Wash Buffer (W1)	3 ml
Elution Buffer (E5) (10 mM Tris-HCl, pH 8.5)	4 ml
PureLink™ Micro Kit Columns (with Collection Tubes)	10 each
PureLink™ Elution Tubes	10 each

PureLink™ Micro Kit Column

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

Part No. 100003663 Rev Date: 8 May 2008

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:	>80%
Primer Removal:	>95%
Processing Time	<6 minutes

Binding Buffer

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

Safety Information

- 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.

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

Preparing Binding Buffer with Isopropanol

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

1. Add 4 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 ethanol as follows:

1. Add 12 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.

Binding Washing and Elution

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

1. To your PCR product, add 4X volume Binding Buffer (B2) with isopropanol (see previous page) per 1X volume PCR product. (e.g. add 200 µl Binding Buffer (B2) with isopropanol to 50 µl PCR product).
2. Vortex to mix thoroughly.
3. Transfer the entire PCR product with Binding Buffer to a PureLink™ Micro 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 (see previous page) to your sample in the PureLink™ Micro Column.
6. Centrifuge at 10,000 × g for 1 minute at room temperature. Discard flow–through and reinsert the PureLink™ Micro 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.
8. Place the PureLink™ Micro Kit Column into an Elution Tube.
9. Add 10 µl Elution Buffer (E1, 10 mM Tris–HCl, pH 8.5) to the center of the PureLink™ Micro Kit Column.
10. Incubate for 1 minute at room temperature.
11. Centrifuge at 14,000 × g for 1 minute to collect the purified DNA into the Elution Tube.
12. Remove and **discard** the PureLink™ Micro Kit Column. The Elution Tube now contains your purified DNA. The recovered elution volume is ~9-10 µl.
13. Store the purified DNA at –20°C for long term or proceed to your downstream application of choice.

Analyzing DNA Yield and Quality

After purification with PureLink™ PCR Micro Kit, the yield of purified DNA 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 4) 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.