Quantum Prep®
PCR Kleen Spin Columns

Catalog Numbers
732-6300 (25 pack)
732-6301 (100 pack)

BIO-RAD

Bio-Rad Laboratories, 2000 Alfred Nobel Drive, Hercules, CA 94547

4006142 Rev B

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Section 1 Introduction

1.1 Intended Use

Quantum Prep PCR Kleen Spin Columns will purify PCR products (≥200 bp) from smaller impurities, which can include unincorporated dNTPs, polymerases, primers, and small primer-dimers. Designed according to Bio-Rad's Micro Bio-Spin column format, these columns provide fast and efficient clean-up of PCR reactions in under 4 minutes. Purified PCR products can be used in ligation reactions, restriction enzyme digests, nested PCR reactions, and labeling reactions.

Note: For cycle sequencing or isolation of a single PCR product from reactions that contain multiple bands, the Quantum Prep Gel Slice Kit should be used (catalog number 732-6160).

Each PCR Kleen spin column is pre-packed with appropriate size exclusion resin in TE buffer, pH 7.4 (10 mM Tris-HCl, 1 mM EDTA, pH 7.4). All column components have been sterilized by autoclaving and each lot is certified to be DNase free. Autoclavable wash and collection tubes are supplied for each column.

1.2 Specifications

The columns are optimized for load volumes of typical PCR reactions (25–100 µl). Smaller sample volumes can be applied to the column, but will result in sample dilution. Volumes greater than 100 µl should be divided into 2 aliquots and purified over 2 columns.

Each lot of PCR Kleen spin columns is tested for recovery and retention of nucleic acid as determined by agarose gel electrophoresis. Recovery of PCR fragments is dependent upon fragment size, with larger fragments yielding higher recoveries than smaller fragments (see sample gel on pg. 6). Typical recoveries range from 85% for ≥700 bp fragments, to 35% for 200 bp fragments. Retention of primer dimers (20 bp) exceeds 95% and retention of primers exceeds 99%, as judged by agarose gel electrophoresis.

Section 2
Technical Information

2.1 Instructions for Use

1. Resuspend the resin in the column by vortexing ~5 seconds.
2. Remove the cap, snap off the tip and place the column in a 2.0 ml wash tube.
3. Pre-spin the column for 1 minute in a microcentrifuge at 735 x g. (If used at higher g forces, results may vary).
4. Place the column in a clean 1.5 ml collection tube.
5. Apply the sample (25–100 µl) to the top center of the column bed, being careful not to disturb the resin (see Figure 1 below).
6. Spin the column for 2 minutes at 735 x g.
7. Save the purified sample which is in the bottom of the 1.5 ml collection tube.
8. Properly dispose of the used column which contains unincorporated dNTP's, primers, and short primer-dimers.

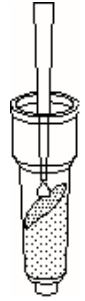

Fig. 1. Application of sample.

2.2 Centrifugation Notes

Quantum Prep PCR Kleen spin columns fit 1.5 ml and 2.0 ml microcentrifuge tubes for sample collection during centrifugation. Use the 2.0 ml capless wash tubes for initial column buffer removal, and the 1.5 ml collection tubes for isolation of purified samples. The tubes which come with the columns are completely autoclavable, and should be autoclaved if sterile tubes are desired.

PCR Kleen spin columns are designed to be used in variable speed bench top microcentrifuges capable of generating a force of 735 x g. The gravitational force created at a particular rpm is a function of the radius of the microcentrifuge rotor. Consult the microcentrifuge instruction manual for conversion information from rpm to g-force. Alternatively, to calculate the speed (rpm) required to reach a gravitational force of 735 x g, use the following equation.

$$RCF\ (g) = (1.12 \times 10^{-5})(rpm)^2 r$$

where r is the radius in centimeters measured from the center of the rotor to the middle of the PCR Kleen column, and rpm is the speed of the rotor in revolutions per minute.

Note: Do not use the pulse button to centrifuge the columns—this overrides the variable speed setting and can cause column failure.

If a variable speed microcentrifuge is not available, PCR Kleen spin columns can be spun in a clinical centrifuge set at 735 x g. Place the column in a collection tube, and set the column and collection tube in a larger tube appropriate for the size of the rotor. Centrifuge at 735 x g for the times specified in the Instructions for Use section.

2.3 Autoclavability

Quantum Prep PCR Kleen spin columns are provided pre-autoclaved and are sterile if unopened. The empty wash and collection tubes should be autoclaved prior to use if sterile tubes are desired.

2.4 Storage and Stability

PCR Kleen spin columns should be stored at 2–8 °C and are stable up to 1 year when stored appropriately. Do not freeze. In older model refrigerators, avoid storing the columns in the rear of a refrigerator where freezing may occur.

2.5 Applications

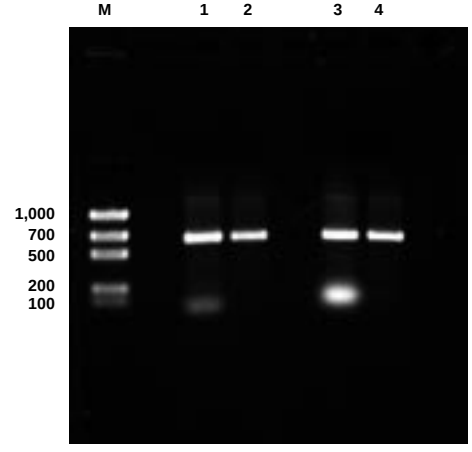

Fig. 2. Removal of primers and primer-dimers from PCR reactions. A PCR reaction mixture containing either 2 µg of a 660 bp fragment and 100 ng of a 25 mer primer-dimer (sample 1) or 2 µg of a 660 bp fragment and 500 ng of a 25 mer primer (sample 3) were purified using PCR Kleen spin columns. 10 µls of each reaction and purified sample were analyzed on a 1.5% agarose gel. Sample 2: primer-dimer purification; sample 4: primer purification; M: DNA markers.

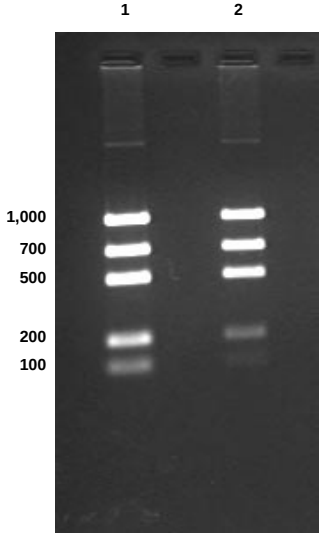

Fig. 3. Recovery of DNA fragments. Recovery of PCR fragments is dependent upon fragment size, with larger fragments giving higher yields than smaller fragments. 2.5 µg of a DNA standard containing 1000, 700, 500, 200, and 100 bp bands was spun through a PCR Kleen spin column. Sample 1: unpurified; Sample 2: column purified.

2.6 Buffer Exchange

The resin in the PCR Kleen spin columns is suspended in 10 mM Tris-HCl, 1 mM EDTA, pH 7.4. The column can be customized to purify a sample in the buffer of choice by performing a buffer exchange. To perform a buffer exchange:

1. Follow steps 1–4 in the Instructions for Use section.
2. Apply the new buffer in 500 µl aliquots. After each application of new buffer, centrifuge (at 735 x g) the column for 30 seconds to remove the buffer. Discard the buffer from the collection tube. Repeat as required. Three washes result in >99% of the buffer exchanged. Four washes result in >99.9% of the buffer exchanged.
3. The sample can now be applied to the column as directed in step 5 in the Instructions section.

Section 3
Quantum and Micro Bio-Spin Products

3.1 Product Information and Applications Guide

Category	Application	Product	Catalog Number
PCR/DNA Fragment Recovery	Purification of single PCR products from PCR reactions with multiple bands (50–4000 bp)	Quantum Prep Gel Slice Kit	732-6160
	Purification of PCR products from dNTPs, primers, and primer-dimers	Quantum Prep PCR Kleen Spin Columns (red caps)	732-6300
	Rapid purification of DNA bands from agarose gel slices	Quantum Prep Freeze-N-Squeeze	732-6165
Plasmid Purification	Purification of plasmids from small scale liquid cultures (1–2 ml)	Quantum Prep Plasmid Miniprep Kit	732-6100
	Purification of plasmids from medium scale liquid cultures (40–60 ml)	Quantum Prep Plasmid Midiprep Kit	732-6120
	Purification of plasmids from large scale liquid cultures (100–500 ml)	Quantum Prep Plasmid Maxiprep Kit	732-6130
Spin-Column Purification of Nucleic Acids	• Removal of unincorporated dNTPs from labeling reactions • Removal of dye terminators from sequencing reactions • Buffer exchange of nucleic acids (> 20 bases or bp)	Micro Bio-Spin 30 Tris Columns (orange caps) Micro Bio-Spin 30 SSC Columns (yellow caps)	732-6223 732-6202