PCR PRODUCT PURIFICATION

UltraClear™ Sequencing Reaction Clean-Up Kit

The UltraClear Sequencing Reaction Clean-Up Kit offers a rapid and simple method for the clean-up of DNA sequencing reactions in a 96-well format. The UltraClear plate uses ultrafiltration membranes to separate low molecular weight contaminants, such as unincorporated dye terminators, dNTPs, and residual salts from the sequencing reaction products.

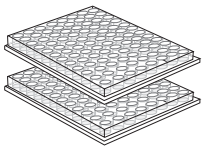
Features and Benefits

- Excellent quality – PHRED q>20 scores greater than 850 bases
- Scientifically engineered to use less BigDye® version 3.1 chemistry and save money
- Optimized centrifugation procedures for maximum flexibility
- Partial 96-well plate can be used and stored at room temperature

Storage: Room Temperature

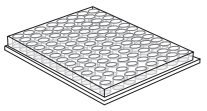
UltraClear Protocol

1. Dilute Sequencing Reactions
2. Load UltraClear Plate



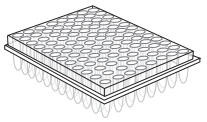
3. Centrifuge

4. Add 40 µl Sequencing Solution



5. Resuspend the Purified Sequencing Products

6. Transfer Sequencing Product to an Appropriate Injection Plate



High Quality Sequence Data with UltraClear Kit

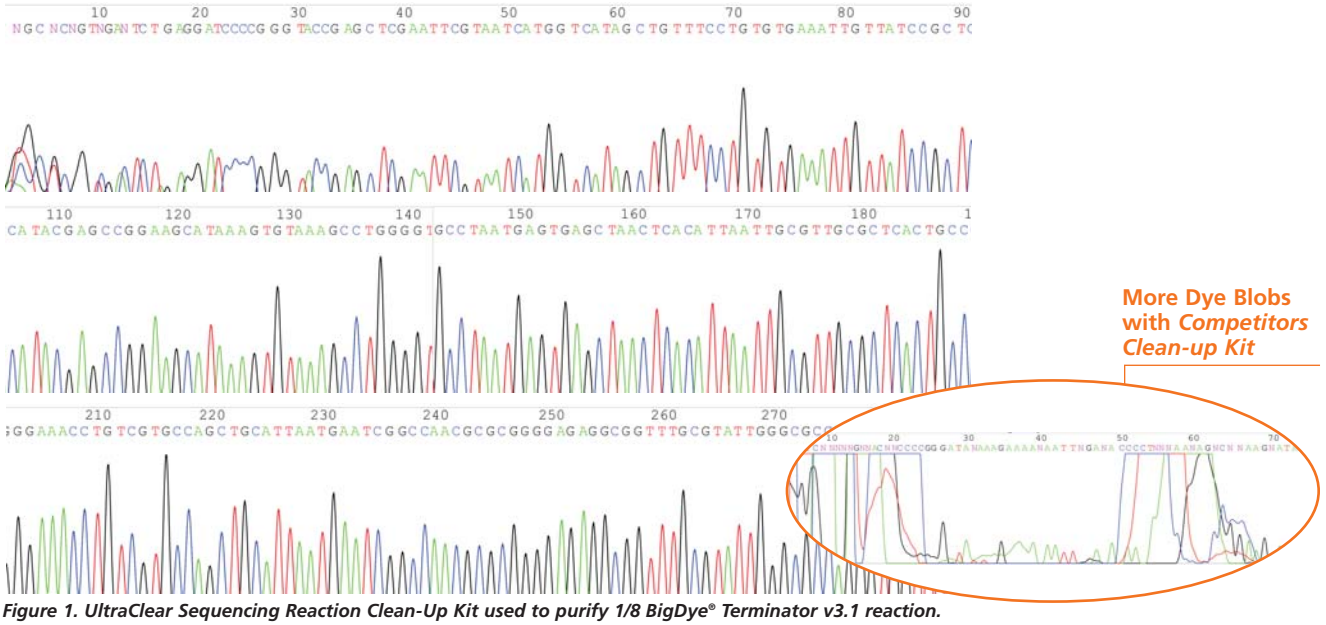


Figure 1. UltraClear Sequencing Reaction Clean-Up Kit used to purify 1/8 BigDye® Terminator v3.1 reaction.

PCR PRODUCT PURIFICATION

Optimized Protocol Accommodates a Variety of Spin Forces and Times

Spin Force	Time	Average PHRED 20	SD
1,000 x g	30 Minutes	887	11
1,500 x g	20 Minutes	870	15
2,500 x g	15 Minutes	880	16
3,500 x g	10 Minutes	859	39

Average PHRED q>20 scores for each spin force/time configuration tested. N=8 for each configuration.

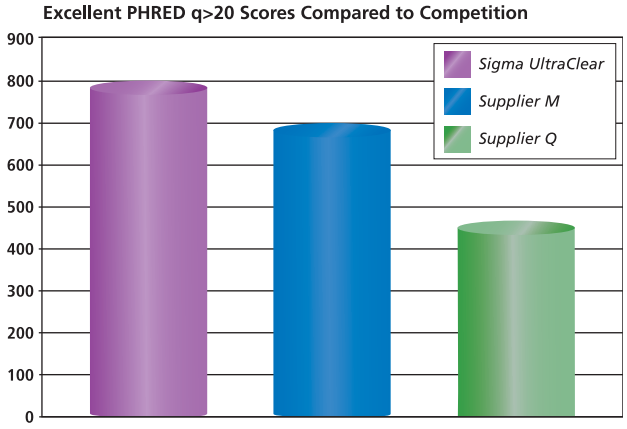


Figure 2. Data quality analysis based on PHRED q>20 scores. *Samples analyzed on the ABI Prism® 3700 under standard run conditions. M13 template was prepared using 1/8 BigDye® Terminator v3.1.*

Cat. No.	Product Description	Quantity
UC9601	UltraClear Sequencing Reaction Clean-Up Kit	1 x 96
UC9604	UltraClear Sequencing Reaction Clean-Up Kit	4 x 96

PCR PRODUCT PURIFICATION

SigmaSpin™ Post-Reaction Clean-Up

For removal of unincorporated dyes, excess salts and other interfering components from sequencing reactions

SigmaSpin Post-Reaction Clean-Up Columns

SigmaSpin Post-Reaction Clean-Up Columns are ideal for lower throughput applications, such as clean-up of probe labeling reactions or small numbers of sequencing reactions.

These columns can accept sample volumes up to 100 µl. Each column comes with a collection tube to collect the DNA during centrifugation.

SigmaSpin 96-Well Post-Reaction Clean-Up Plates

SigmaSpin 96-Well Post-Reaction Clean-Up plates provide a fast, simple, and highly efficient method for removing unincorporated dyes, excess salts, and other interfering reaction components (Fig. 1). Each plate is packed with a pre-hydrated size-exclusion resin, equilibrated with molecular biology grade water, and supplied in our unique plate design with long drip directors to minimize contamination between samples. Our new plate design also includes a snap-cap bottom seal and a foil seal on top of the plate to ensure that the resin remains hydrated. SigmaSpin has been tested in high-throughput genome centers and core facilities with ABI Prism® 3700, 3100 and 377. Each well can accept sample volumes up to 20 µl with DNA typically eluted in a volume of 20-25 µl.

Ideal for removing

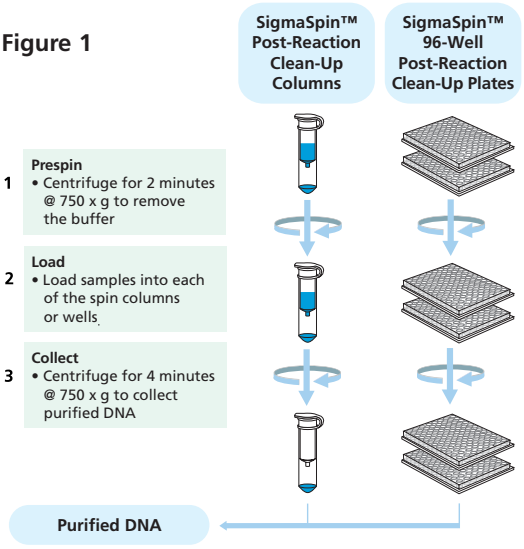
- Dye-terminator nucleotides and primers from sequencing reactions
- Radiolabeled nucleotides, primers, and fluorescent dyes from nucleic acid probe labeling reactions

Features and Benefits

- Validated with all automated sequencers and all dye terminators including BigDye® v. 3.0
- Pre-qualified size-exclusion resin guarantees optimum performance
- Unique drip directors prevent cross-contamination between samples during collection
- Plates are sealed to eliminate leakage or drying during shipping or storage
- Suitable for use with multi-channel pipettes and automated workstations

Storage: 2-8 °C

Figure 1



Use of a SigmaSpin Post-Reaction Clean-Up Plate results in improved read quality

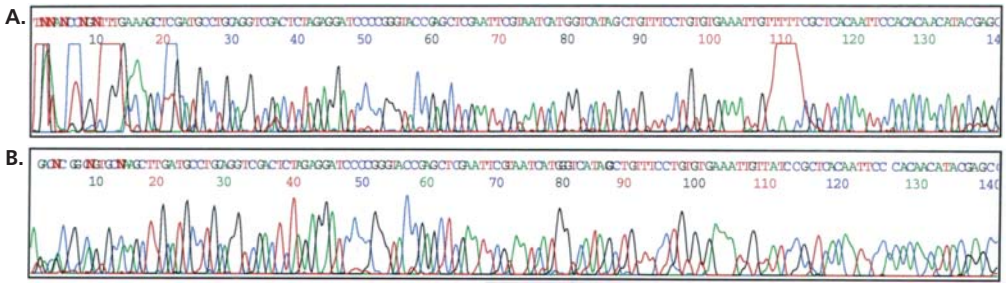


Figure 2. *Single stranded M13MP18 plasmid was sequenced with a -21M13 forward sequencing primer using ABI BigDye® Terminator chemistry. Sequencing reactions were resolved on an ABI Prism® 377 XL instrument with a 48 cm gel cassette containing 4.5% AutoPAGE™ Plus acrylamide at 2.88kV for 7 hrs.*

Panel A: *Sequencing reactions were precipitated with 70% ethanol and placed on ice for thirty minutes. DNA pellets were dried and resuspended in TE solution prior to electrophoresis.*

Panel B: *Sequencing reactions were subjected to post-reaction clean-up with SigmaSpin™ Post-Reaction Clean-Up 96-Well Plates, according to recommended protocol.*

Note the removal of dye blobs and the increase in base calling accuracy with SigmaSpin™ compared to ethanol precipitation.

Cat. No.	Product Description	Quantity
S5059	SigmaSpin Post-Reaction Clean-Up Columns (with collection tubes)	70 each
S4309	SigmaSpin 96-Well Post-Reaction Clean-Up Plates*	2 each
S4434	SigmaSpin 96-Well Post-Reaction Clean-Up Plates*	10 each
S4559	SigmaSpin 96-Well Post-Reaction Clean-Up Plates	50 each
P4736	48-Well Wash Plate	50 each
Z374903	96-Well Collection Plate	2 pkg (25/pkg)

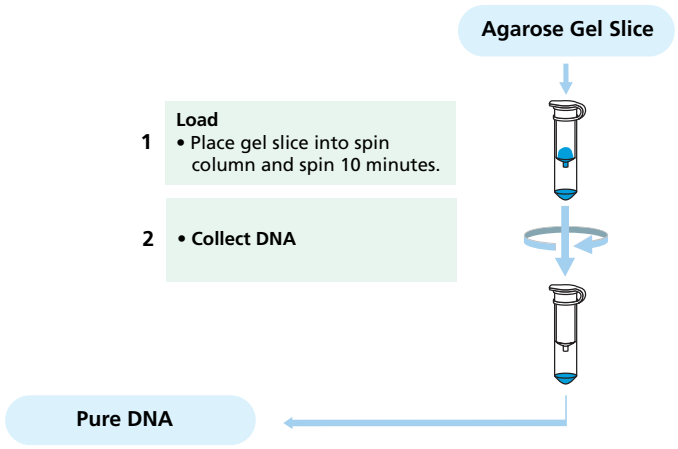
**Wash and collection plates included in 2- and 10-each package sizes*

PCR PRODUCT PURIFICATION

GenElute™ Agarose Spin Columns

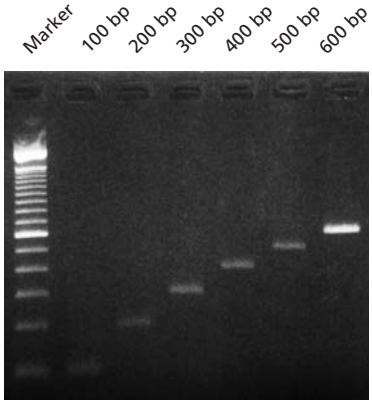
For purification of DNA from agarose gel slices

GenElute Agarose Spin Columns isolate DNA by centrifugation/filtration. A slice of agarose gel containing the DNA of interest is excised, placed into a spin column, and spun at maximum speed in a microcentrifuge. Agarose is retained on a filter in the spin column and soluble molecules, including DNA, pass through the filter and collect in a microcentrifuge tube.



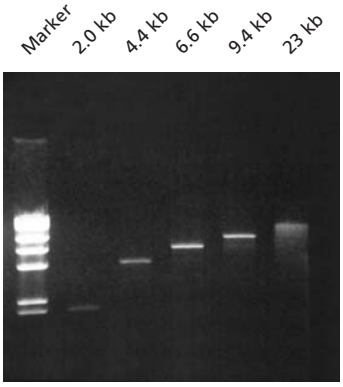
Features and Benefits

- One-step, 10 minute protocol
- No melting or digestion of agarose required
- Simpler and quicker than bind and elute or electroelution methods
- No ethanol precipitation
- Typical recovery of 40 to 45% for DNA fragments from 100 bp to 10 kb (Figs. 1 and 2)



Recovery of 100-600 bp DNA fragments purified using GenElute Agarose Spin Columns

Figure 1. *1 µg of 100 bp DNA ladder was electrophoresed on a 2.0% agarose gel. 100, 200, 300, 400, 500, and 600 bp fragments were excised from the gel, and purified using GenElute™ Agarose Spin Columns as above. Samples were loaded and analyzed on a 2.0% agarose gel.*



Recovery of 2 to 23 kb DNA fragments purified using GenElute Agarose Spin Columns

Figure 2. *Lambda* Hind *III markers were pre-heated at 65 °C and electrophoresed on a 2.0% agarose gel. The 2.0, 4.4, 6.6, 9.4, and 23 kb fragments were excised and purified using GenElute™ Agarose Spin Columns. Samples were loaded and analyzed on a 2.0% agarose gel.*

GenElute™ Minus EtBr Spin Columns

For recovering DNA from ethidium bromide (EtBr) stained agarose gels or EtBr-containing solutions

GenElute Minus EtBr Spin Columns are based on the GenElute Agarose Spin Column but incorporate a membrane for the selective removal of ethidium bromide (Fig. 1). The DNA band is excised from an agarose gel and loaded onto the spin column. The membranes, embedded within the column, retain agarose and ethidium bromide while allowing DNA to selectively pass through the column into a centrifuge tube during centrifugation.

Up to 95% of ethidium bromide is removed from DNA with a simple 10 minute procedure. GenElute Minus EtBr Spin Columns can recover DNA fragments from 100 bp to 10 kb with typical recoveries of 30 to 35%. The purified DNA can be used directly for ligation, restriction enzyme digestion, cloning, PCR and related manipulations without ethanol precipitation.

Features and Benefits

- Removes up to 95% of EtBr from DNA
- One-step, 10 minute protocol
- No melting or digestion of agarose required
- Simpler and quicker than bind and elute or electroelution methods
- No ethanol precipitation

Ethidium Bromide removal demonstrated over a UV light box

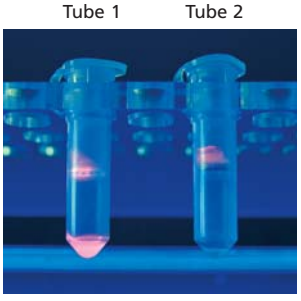


Figure 1. *DNA was electrophoresed in agarose gels containing ethidium bromide (0.5 µg/ml). Bands were excised, applied to both the Minus EtBr and Agarose Spin Columns and centrifuged. The final eluate from each column was compared under a UV light box. The filter within the GenElute™ Minus EtBr Spin Column retained ethidium bromide.*

Tube 1: *GenElute Agarose Spin Column*
Tube 2: *GenElute Minus EtBr Spin Column*

Cat. No.	Product Description	Quantity
56500	GenElute Agarose Spin Columns	70 each
56501	GenElute Minus EtBr Spin Columns	70 each

PCR PRODUCT PURIFICATION

GenElute™ PCR Clean-Up Kit

For purification of single- or double-stranded PCR amplification products

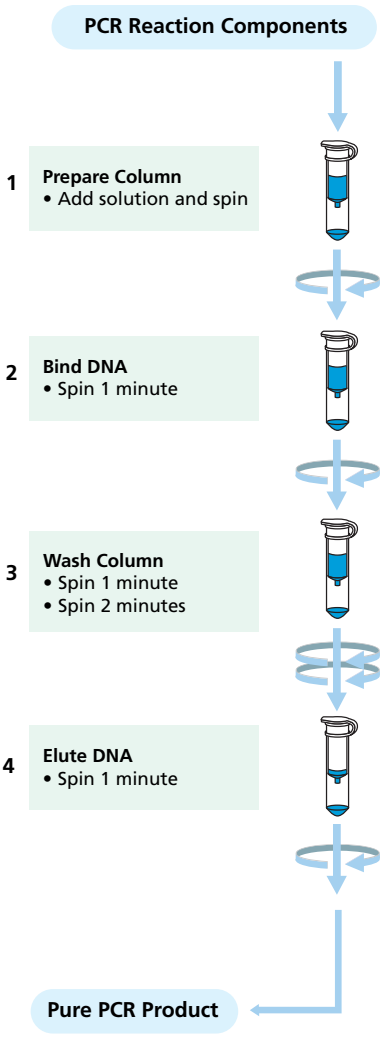
The GenElute PCR Clean-Up Kit is designed for rapid purification of single-stranded or double-stranded PCR amplification products (100 bp to 10 kb) from other components in the reactions, such as excess primers, nucleotides, DNA polymerase, oil and salts. This kit combines the advantages of silica binding with a convenient spin column format, eliminating the need for expensive resins or toxic organic compounds such as phenol and chloroform.

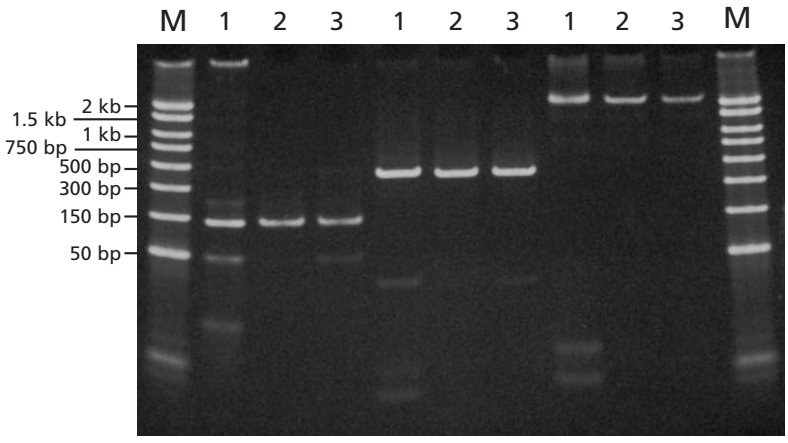
DNA is bound on a silica membrane within the spin column. The bound DNA is washed and the clean, concentrated DNA is eluted in the buffer of choice. Each column can purify up to 100 µl or 10 µg of PCR amplified DNA and recover up to 95% of PCR products between 100 bp and 10 kb. More than 99% of the primers and most primer-dimers (<40 bp) are removed. Purified DNA can be used in enzymatic reactions, conventional or automated sequencing, cloning and microarray analysis.

Features and Benefits

- Purifies up to 100 µl or 10 µg of PCR amplified DNA in 5 minutes
- Recovers up to 95% of PCR products between 100 bp and 10 kb
- Removes over 99% of primers and other components
- Eliminates the need to remove mineral oil by organic extraction
- 40% more purification preps supplied than market leader

Storage: Room Temperature
R: 11-22-36/37/38-67 S: 7-16-24/25-26-36





Comparison of PCR product recovery and primer removal

Figure 1. *Three separate PCR products were purified with GenElute PCR Clean-Up Kit and the kit from Supplier Q. Products were 143 bp from corn leaf, 375 bp from pBR322, and 2 kb from human blood. Samples were analyzed on a 20% TBE acrylamide gel and visualized by staining with SYBR® Green II.*

Lanes 1: Unpurified Reaction
Lanes 2: GenElute PCR Clean-Up Kit
Lanes 3: Supplier Q Kit

Cat. No.	Product Description	Purifications	Quantity
NA1020	GenElute PCR Clean-Up Kit	70	1 kit