

Sequencing

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
- Partial 96-well plate can be used and stored at room temperature
- Optimized centrifugation procedures for maximum flexibility
- Optimized for use with the X-Tractor™ Gene liquid handling system. For additional information, please visit www.corbetrobotics.com

Storage: Room Temperature

UltraClear Protocol

1 Dilute Sequencing Reactions

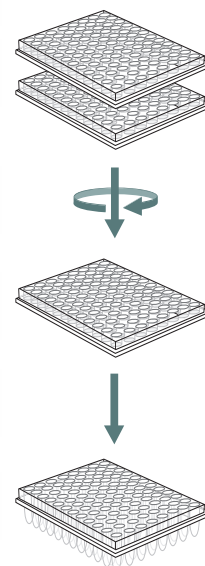
2 Load UltraClear Plate

3 Centrifuge

4 Add 40 μ l Sequencing Solution

5 Resuspend the Purified Sequencing Products

6 Transfer Sequencing Product to an Appropriate Injection Plate



High Quality Sequence Data with UltraClear Kit

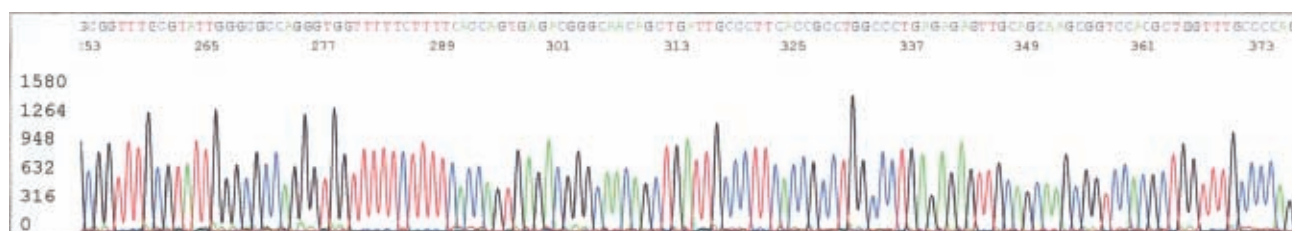


Figure 1. UC9601 UltraClear Sequencing Reaction Clean-up kit. Single-stranded M13MP18 Phage DNA was sequenced with -21M13 forward primer utilizing Applied Biosystems BigDye Terminator v 3.1 chemistry. The reaction was prepared with 1/2 volume of BigDye Terminator and 5x dilution buffer. The sequencing reaction was cleaned using the UltraClear Sequencing Clean-up kit. Analysis was performed on an ABI 3730xl with a 36 cm capillary array with POP7 polymer.

Excellent PHRED $q > 20$ Scores Compared to Competition

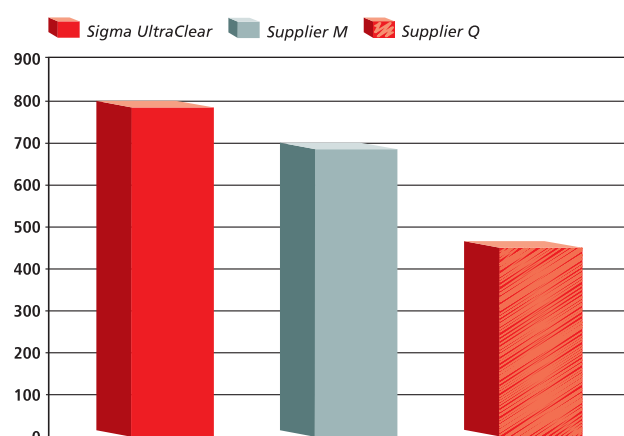


Figure 2. Data quality analysis based on PHRED $q > 20$ scores. Samples analyzed on the ABI Prism® 3700 under standard run conditions. M13 template was prepared using 1/8 BigDye® Terminator v3.1.

Optimized Protocol Accommodates a Variety of Spin Forces and Times

Spin Force	Time	Average PHRED 20	SD
1000 x g	30 Minutes	887	11
1500 x g	20 Minutes	870	15
2500 x g	15 Minutes	880	16
3500 x g	10 Minutes	859	39

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

Ordering Information

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

Sequencing

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.

Ideal for removing

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

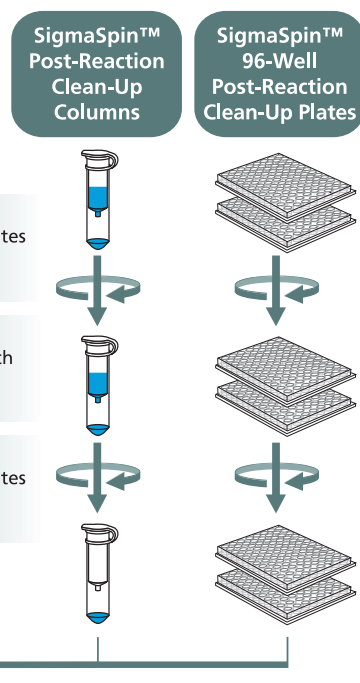
Features and Benefits

- Validated with many common automated sequencers and dye terminators
- 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

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. The plate design also includes a snap-cap bottom seal and a foil seal top to ensure that the resin remains hydrated. SigmaSpin has been tested in high-throughput genome centers and core facilities with ABI Prism® 3700, 3100, 310, and 377. Each well can accept sample volumes up to 20 µl.

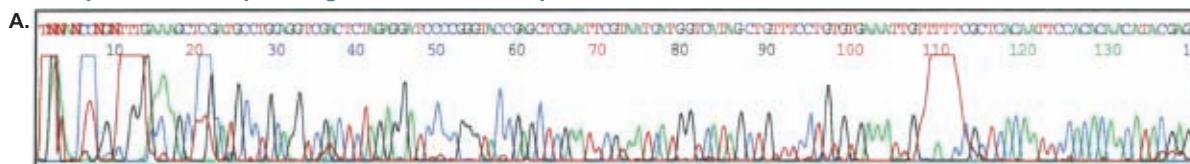
Storage: 2-8 °C



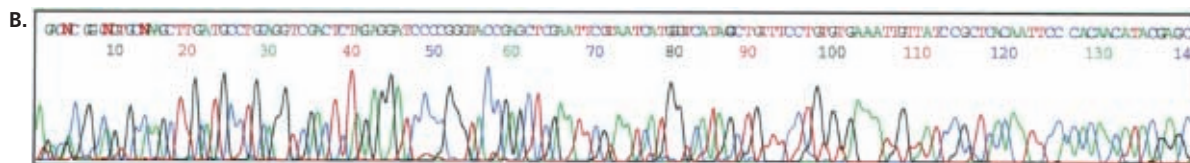
SigmaSpin Post-Reaction Clean-Up technology comes in two convenient forms, 96-well plates and single spin columns. Each format comes ready for immediate use.

Sequencing

Comparison of Sequencing Reaction Clean-Up Methods



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.

Figure 1. 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 AutoPAGE™ Plus 4.5% acrylamide at 2.88kV for 7 hrs.

SigmaSpin Efficiently Removes Interfering Reaction Components for Long and Accurate Reads

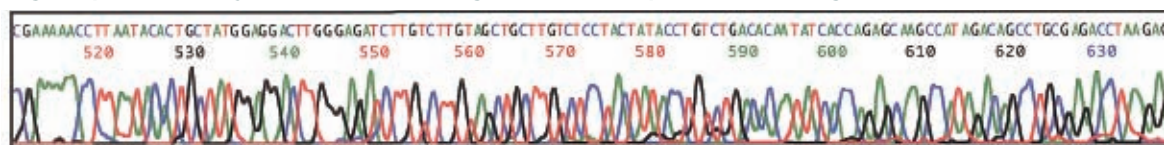


Figure 2. Sequencing reaction was purified using a SigmaSpin 96-well plate. pFLAG-MAC™ plasmid (Cat. No. E5644) was sequenced using BigDye® Terminator v 3.0 chemistry (20 µl total reaction volume; BigDye® premix diluted 1:1 with SeqSaver™ [Cat. No. S3938]). Data was generated on an ABI Prism® 3700 DNA Analyzer with POP-6™ polymer and 10X CE Buffer (Cat. No. B4930). PHRED g20 > 600.

Ordering Information

Cat. No.	Product Description	Quantity
S5059	SigmaSpin™ Post-Reaction Clean-Up Columns (with collection tubes)	70/pkg
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	Collection plate, 1.5 ml x 48 wells	50 each
Z374903	96-Well Collection Plates (PCR Multiwell Plates)	2 pkg (25/pkg)

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

Sequencing

10X CE Buffer

Sigma's 10X Capillary Electrophoresis Running Buffer was designed to meet the high quality and high throughput demands of today's sequencing labs. As a primary manufacturer and leading supplier of TBE to the gel-based sequencing community Sigma has the knowledge and large scale capacity to meet your buffer needs. Scientists at Sigma have taken this experience and joined it with today's advancing technologies to optimize a buffer for use with capillary electrophoresis sequencing instruments.

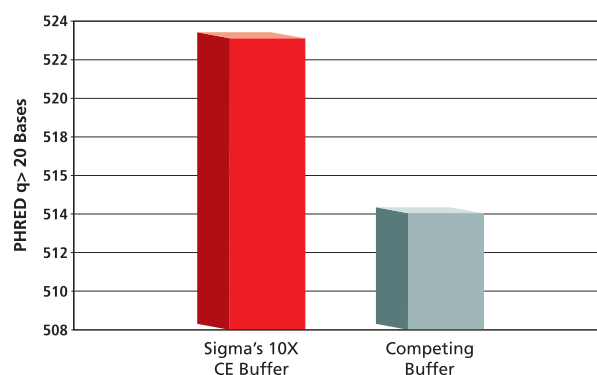
Features and Benefits

- Cost efficient – high quality buffer at a great value
- Quality control testing of every lot ensures proper pH, conductivity and performance for capillary electrophoresis sequencing
- Compatible with ABI Prism® 3700, 3100, and 310 DNA sequencers
- Room temperature stability for convenient shipping and storage
- Plastic bottles provide easy handling
- 1, 4, and 20 L as well as custom packaging options available

Storage: Room Temperature



PHRED q> 20 Scores
Sigma's 10X CE Buffer vs. Competitors



Data quality analysis based on PHRED q>20 scores. Average read length of both single-stranded and double-stranded DNA samples were analyzed on the ABI Prism® 3700 under standard run conditions 3 hours at 1,000V, 50 °C. Samples were prepared using BigDye® terminators with SigmaSpin™ post-reaction clean up.

10X CE Buffer Performance

	Run Time	Average PHRED q>20 bases	Average total read length
ABI Prism® 3700	3.0 (h)	520	800
MegaBACE™	2.75 (h)	495	760

Data quality analysis based on PHRED q>20 scores and total readable bases. Data was generated from M13, pGEM, and pbluescript templates using BigDye® Terminators and standard run conditions.

Ordering Information

Cat. No.	Product Description	Quantity
B4930	10X Capillary Electrophoresis Running Buffer	1 liter 4 liter 20 liter

Sequencing

SeqSaver™

A universal dilution buffer for all common reaction premix chemistries – including BigDye® v. 3.0

SeqSaver Sequencing Premix Dilution Buffer is designed to decrease the overall cost of sequencing by allowing the user to decrease the amount of reaction premix needed for each sequencing reaction. SeqSaver is a universal sequencing reaction diluent compatible with all commonly available reaction premixes including BigDye® (v. 1.0, 2.0, and 3.0), ThermoSequenase™ II, and standard terminator premixes.

Sequencing data obtained using SeqSaver in a 1:1 ratio with reaction premix yields resolution, read length, and accuracy identical to unmodified reaction mixes. Strict QC and performance testing eliminates lot-to-lot variation resulting in consistent results every run. SeqSaver is suitable for analysis using ABI Prism® gel-based or capillary sequencing instruments and Amersham Biosciences MegaBACE™. With additional optimization some templates allow for further dilution and reduced total reaction volumes for even greater savings.

Features and Benefits

- Can be used with BigDye® (v. 1.0, 2.0, and 3.0), ThermoSequenase™ II, DYEnamic™ ETs, and other standard terminator premixes
- Reduces the amount of costly reaction premix by 50% or more
- No compromise in read length, resolution or signal strength
- SeqSaver can be mixed with individual reactions or directly to your stock premix
- Can be used with reaction volumes of 10 µl or less
- Custom packaging options available for large scale use

Storage: -20 °C

R: 36/37/38 S: 26-36

Sequencing data obtained using SeqSaver™ in a 1:1 ratio with reaction premix yields resolution, read length and accuracy identical to unmodified reaction mixes.

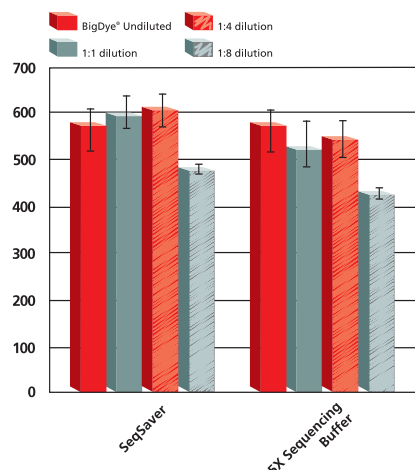


Figure 1. Data quality analysis based on PHRED q>20 scores. Comparison of sequencing reactions generated with dilutions of reaction premix using SeqSaver and a 5X Sequencing buffer. Data was generated from M13MP18 and pGEM templates using BigDye® terminators and SigmaSpin™ post-reaction clean-up plates. Reaction premix was diluted 1:1, 1:4, and 1:8 in a 20 ml reaction and compared to undiluted reaction premix control.

Decrease the cost of sequencing without compromising signal strength, read length, or data quality!

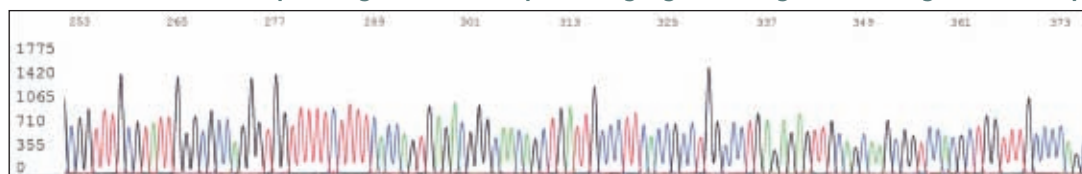


Figure 2. BigDye Terminator v 3.1 utilizing a 1:1 ratio of BigDye Terminator and SeqSaver. The data was generated from M13MP18 Phage DNA and -20 M13 forward primer. Samples were analyzed on Applied Biosystems 3730xl with a 36 cm array and POP7 polymer.

Ordering Information

Cat. No.	Product Description	Reactions (20 µl rxn)	Reactions (10 µl rxn)	Quantity
S3938	SeqSaver™ Sequencing Premix Dilution Buffer	100	200	0.4 ml
		500	1000	2 x 1 ml
		1000	2000	4 x 1 ml
		5000	10000	20 ml

Custom packaging options are available – inquire for more information.