

Montage® Sequencing Reaction Cleanup

Membrane-based size-exclusion filter plates deliver quality sequencing results

- ▶ 10-minute vacuum-based protocol
- ▶ Compatible with a variety of templates
- ▶ Optimized for use with BigDye® chemistries
- ▶ 96- and 384-well formats available
- ▶ Automation compatible

Fast, Vacuum-based Protocol

Montage SEQ Sequencing Reaction Cleanup kits incorporate patented size-exclusion technology to yield highly purified sequencing reaction products. In addition to eliminating centrifugation steps, Montage kits do not require filtrate collection or column packing.

The 96- and 384-well filter plates follow a vacuum-driven protocol and are designed for use with automated DNA sequencers, including Applied Biosystems Prism® and Amersham Biosciences MegaBACE® systems.

Improved Consistency

Size-exclusion purification eliminates variability in sequencing reaction cleanup. Since the membrane-based protocol does not require alcohol

precipitation, there is no risk of salts or ethanol affecting final sequencing results. In addition to high pass rates, highly reproducible results are obtained with PCR and plasmid templates prepared by a variety of methods.

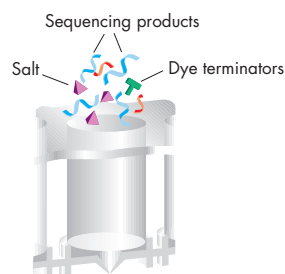
More Usable Data from Each Sequencing Reaction

Montage kits are optimized for use with BigDye chemistries (including v1.1 and 3.1) and are developed to save reagents. The protocol recommends 1/8x scale reactions to save on reagent costs, reduce dye blobs, and provide for more usable sequencing data.

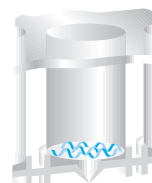
Millipore protocols are thoroughly tested and are consistent with ABI recommendations.

Patented Size-exclusion Technology

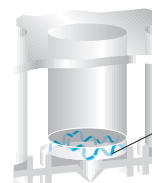
Montage SEQ Sequencing Reaction Cleanup kits follow a 10-minute, vacuum-driven protocol that is easily integrated with automation.



1. Introduce samples into wells, add injection solution.



2. Filter using Montage vacuum manifold until wells are empty. DNA is retained on the membrane surface while smaller contaminants are filtered to waste.



3. Wash sample once, resuspend, and recover.

Purified sequencing products

Montage SEQ₉₆ Sequencing Reaction Cleanup Kit with Plasmid Template

ABI 3100, 1/8x BigDye v3.1

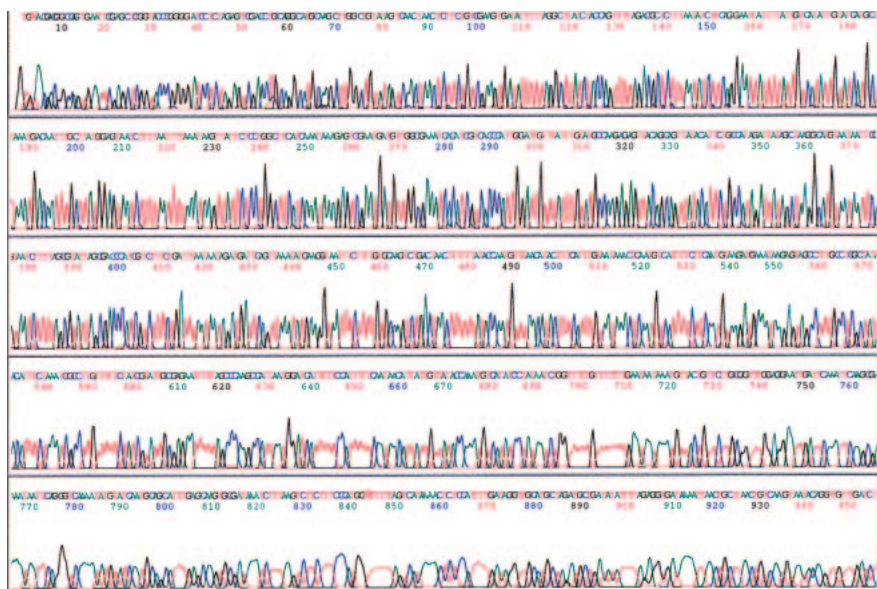


Figure 1. Plasmid DNA (pLH-2, 200 ng), purified using a Montage Plasmid Miniprep₉₆ kit, was sequenced in a 1/8x BigDye Terminator v3.1 reaction. After purification using a Montage SEQ₉₆ Sequencing Reaction Cleanup kit, the samples were analyzed on an ABI Prism 3100 Avant capillary sequencer (80 cm capillary). This electropherogram is typical of the high quality reads achieved using this cleanup method (Phred 20 = 882).

Montage SEQ₉₆ Sequencing Reaction Cleanup Kit with PCR Template

ABI 3700, 1/8x BigDye v1.1

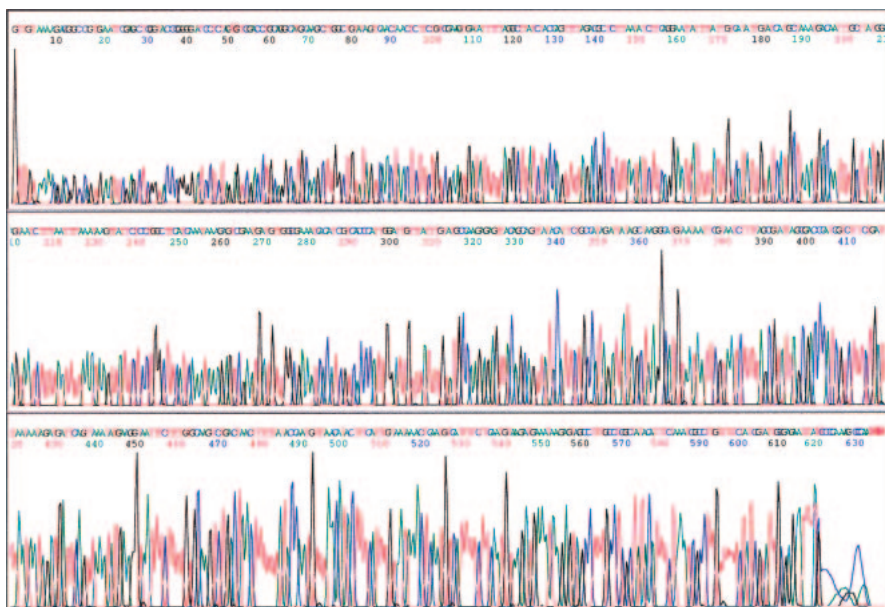


Figure 2. A 657 bp PCR product (10 fmole) was purified using Montage PCR₉₆ filter plate and sequenced in a 1/8x BigDye Terminator v1.1 reaction. After purification using the Montage SEQ₉₆ Sequencing Reaction Cleanup kit, the samples were analyzed on an ABI Prism 3700 DNA sequencer. Using this approach, the first base after the primer is readily detectable.

Faster Protocols, Improved Results

Method	Process Time	Typical Phred 20 Values	Average Pass Rates
Montage SEQ ₉₆ Sequencing Reaction Cleanup kit	10 minutes	680	>97
Brand Q 96-well gel filtration-based dye terminator removal system*	55 minutes	630	94

*Protocol as per manufacturer recommendations.

Table 1. Comparison of sequencing reaction cleanup methods: Sequencing was done on plasmid templates, 1/8x BigDye reactions, and run on the ABI Prism 3700. Results show that the Montage SEQ₉₆ Sequencing Reaction Cleanup kit delivers long read lengths and high pass rates in less time than gel filtration-based systems.

Optimized for use with BigDye Chemistries

Long read lengths and high pass rates for multiple reagent concentrations

BigDye Version	Reaction Size [†]	Pass Rate (%) Phred 20 >300	Phred 20 Score (ABI 3700)	CV (%)
3.1	1/8*	97	741	6
	1/4*	94	709	10
1.1	1/8	96	695	10
	1/4	96	690	6

*Montage Sequencing Wash Solution (Catalog no. LSKS BW5 00) is required for sequencing reactions >1/4 x to minimize the formation of dye blobs. For 1/8x reactions, use the Montage Sequencing Wash Solution if dye blobs are observed.

[†]Millipore recommends 1/8x reaction scale for optimal results.

Table 2. Plasmid DNA template purified by the Montage SEQ₉₆ Sequencing Reaction Cleanup kit was analyzed on an ABI Prism 3700 DNA Sequencer (n = 96 for 1/8x reactions, n = 48 for 1/4x reactions).

Montage Wash Solution Reduces Dye Blobs

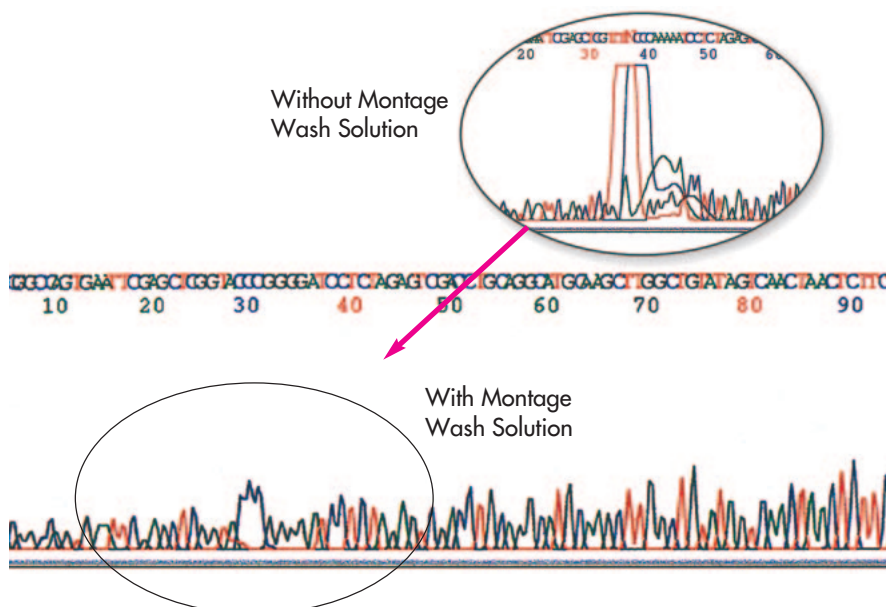


Figure 3. Montage SEQ₉₆ Sequencing Reaction Cleanup kit with optimized wash solution effectively reduces dye blobs that may be generated in sequencing reactions.

Ordering Information

Description	Qty/Pk	Catalogue No.
Montage SEQ ₉₆ Sequencing Reaction Cleanup Kit (includes 96-well filter plates and injection solution)	1 x 96 wells	LSKS 096 01
	4 x 96 wells	LSKS 096 04
	24 x 96 wells	LSKS 096 24
Montage SEQ ₃₈₄ Filter Plates (does not include reagents)	10 plates	S384 SEQ 10
	50 plates	S384 SEQ 50

Accessories

Description		Catalogue No.
Montage Wash Solution, 500 mL		LSKS BW5 00
Montage Injection Solution, 500 mL		LSKS IS5 00
Vacuum Manifold for Use with Montage SEQ ₉₆ and SEQ ₃₈₄ Filter Plates		SAVM 384 01
Portable Vacuum Pump	115 V, 60 Hz	WP61 115 60
	220 V, 50 Hz	WP61 220 50
	100 V, 50 – 60 Hz	WP61 100 60
Vacuum Filtering Flask, 1 L		XX10 047 05
Millex®-FG ₅₀ Filter Units, 10/pk		SLFG 050 10
Silicone Tubing for Vacuum Use, 3/16" I.D., 1.4 m		XX71 000 04
Stopper, No. 8, perforated silicone		XX10 047 08

Related Products

Montage BAC₉₆ Miniprep Kit
Montage Plasmid Miniprep₉₆ Kit
Montage PCR₉₆ Filter Plate
Montage PCR₉₆ Filter Plate
Montage PCR₃₈₄ Filter Plate

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