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Montage SEQ$_{96}$ Sequencing Reaction Cleanup Kit

User Guide

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Contents

Introduction

The Montage SEQ_{96} Sequencing Reaction Cleanup Kit provides the filtration plate and solution necessary to remove contaminating salts and unincorporated dye terminators from DNA sequencing reactions. The vacuum-based size exclusion separation platform eliminates the need for gel filtration or bind-wash-elute columns that require centrifugation. The entire protocol requires very little time to process — typically less than 10 minutes. In addition, the optimized filtration area enables reaction miniaturization and reagent reduction, resulting in lower DNA sequencing costs.

Samples prepared with the Montage PCR_{96}, Montage $PCR_{\mu 96}$ and Montage Plasmid $Miniprep_{96}$ products provide the cleanest templates for DNA sequencing reactions. The Montage SEQ_{96} Sequencing Reaction Cleanup Kits are compatible with ABI Prism® BigDye™ or Amersham DYEnamic™ ET Dye Terminator chemistries and are designed for use with a variety of automation platforms.

Kit Components

The following parts are included in the Montage SEQ_{96} Sequencing Reaction Cleanup Kit:

- 96 well SEQ plate
- Injection Solution

Additional Equipment Required

The user must supply the following equipment in order to use the Montage SEQ_{96} Sequencing Reaction Cleanup Kit. **See the Ordering Information section for details about obtaining these items.**

- Millipore vacuum manifold
- 3/16" I.D. silicone tubing, for vacuum use
- Vacuum filtering flask, 1L
- No. 8 perforated silicone stopper
- Vacuum/pressure pump, 115 V/60 Hz, 220 V/50 Hz, or 100 V/50–60 Hz
  NOTE: Use of house vacuum is not recommended.
- Millex®-FG_{50} filter, for vacuum line protection
- Plate shaker or liquid handler capable of providing agitation of reconstituted samples or multichannel pipettor

Additional Equipment Required, continued

- Capillary sequencer manufacturer's injection plate
- (Optional) Montage Sequencing Wash Solution for reduction of dye blobs under certain conditions — See recommendations in Table 3 on page 9.

Protocol Guidelines

- Montage kits contain disposable, single-use-only plates.
- This kit is for research use only. Not for use in clinical applications.
- Operate at ambient temperature.
- Not for use in centrifugal mode.
- For use with vacuum only.
- Filtration time varies depending on volume added to the wells and the strength of the vacuum source. It is important that all wells are completely emptied of liquid before resuspending purified sequencing reactions.
- If dye blobs become a problem, the reduction of dye blob intensity can be achieved by using the Montage Sequencing Wash Solution for the dilution and rinse steps of the purification process.

Storage Conditions

This kit should be stored at 15 °C to 30 °C.

Procedure for DNA Sequencing Reaction Cleanup

This protocol is based on the following reaction scale. A 1x reaction always uses 8 µL of BigDye Terminator Ready Reaction Mix (BDT), regardless of the final reaction volume.

1/2 reactions = 4 µL BDT
1/4 reactions = 2 µL BDT
1/8 reactions = 1 µL BDT

NOTE: For optimal performance, a 1/8x reaction scale is recommended and is assumed for the following procedure.

1. Set up sequencing reactions in a thermal cycling plate based on information in Table 1.
2. Amplify samples using an appropriate thermal cycling program.
3. Dilute sequence reactions by adding 20 µL of Injection Solution. Mix gently by pipetting up and down 3–5 times.
4. Transfer diluted reactions from the thermal cycling plate into the bottom of SEQ_{96} plate wells.
5. Place the SEQ_{96} plate on the vacuum manifold.

Table 1. Sequencing Reaction Setup — 1/8x

Final Reaction Volume	**5.0 µL**	**10.0 µL**
Template[1,2]	2.0 µL	2.0 µL
Plasmid (150–400 ng/well)		
PCR (10–50 fmol/well)		
ABI 5x Sequencing Buffer	0.5 µL	1.5 µL
Primer (5 pmol/µL)	1.0 µL	1.0 µL
BDT Premix	1.0 µL	1.0 µL
Milli-Q® Water[3]	0.5 µL	4.5 µL

[1] *Template quality has the most dramatic effect on sequencing quality. For optimal results, Millipore recommends that plasmid and PCR templates be prepared with Montage Kits.*

[2] *The suggested DNA template amounts represent general guidelines based on a standard 4.7 kb plasmid. Optimization may be required to accommodate plasmids of other sizes.*

[3] *BigDye mix, primer, 5x Sequencing Buffer and water are typically mixed together to make a premix sequencing cocktail. An appropriate amount of cocktail is then dispensed into each well.*

Procedure for DNA Sequencing Reaction Cleanup, continued

6. Set the vacuum to 23–25" Hg.
7. Apply vacuum until the solution has been completely removed from the wells (2–3 minutes). Continue to apply vacuum for 15 to 30 seconds after the last well is empty.
8. Shut off the vacuum source and remove the SEQ_{96} plate from the manifold.
9. Blot the excess liquid from the bottom of the SEQ_{96} plate by briefly pressing the plate on an absorbent material such as paper towels.
10. Add 20 µL of Injection Solution into the bottom of each well.
11. Place the SEQ_{96} plate on the vacuum manifold and apply vacuum until the solution has been completely removed from the wells (3–4 minutes). Continue to apply vacuum for 15 to 30 seconds after the last well is empty.
12. Shut off the vacuum source and remove the SEQ_{96} plate from the manifold.
13. Blot the excess liquid from the bottom of the plate by briefly pressing the plate on an absorbent material such as paper towels.
14. Add 20 µL of Injection Solution into the bottom of each well of the SEQ_{96} plate.

Procedure for DNA Sequencing Reaction Cleanup, continued

15. Resuspend the purified sequencing products in the Injection Solution by pipetting up and down 20 times with an automated liquid handler. Alternatively, the DNA can be resuspended by shaking for 10 minutes on a microplate shaker. (See "Optimizing Plate Shaker for Sample Resuspension" section for further information.)
16. Transfer the purified sequencing products to an appropriate injection plate.
17. Samples should be injected at 2 kV for 15 seconds into the ABI Prism 3700 sequencer. For the 3730, 3730XL, 3100 and 3100 Avant instruments, use the injection parameters supplied by ABI.

Alternative Sequencing Reaction Setups

If a 1/2x or 1/4x reaction scale is being used, set up sequencing reactions in a thermal cycling plate based on information in Table 2. Then, follow the standard "Procedure for DNA Sequencing Reaction Cleanup" as described in the previous section. However, it may be necessary to use 30 µL of Montage Sequencing Wash Solution in steps 3 and 10 of the procedure instead of 20 µL of Injection Solution. Millipore recommends the use of Montage Sequencing Wash Solution as defined in Table 3.

Table 2. 10 µL Sequencing Reaction Setups — 1/4x and 1/2x

Reaction Scale	**1/4x**	**1/2x**
Template *Plasmid (150–400 ng/well)* *PCR (10–50 fmol/well)*	2.0 µL	2.0 µL
ABI 5x Sequencing Buffer	1.0 µL	0 µL
Primer (5 pmol/µL)	1.0 µL	1.0 µL
BDT Premix	2.0 µL	4.0 µL
Milli-Q Water	4.0 µL	3.0 µL

Table 3. Recommendations for Use of Montage Sequencing Wash Solution

Reaction Scale	1/2x	1/4x
BDT Version 1.1		
Step 3	Wash Solution (30 µL)	Injection Solution (20 µL)
Step 10	Wash Solution (30 µL)	Injection Solution (20 µL)
Injection	Injection Solution (20 µL)	Injection Solution (20 µL)
BDT Version 3.1		
Step 3	Wash Solution (30 µL)	Wash Solution (30 µL)
Step 10	Wash Solution (30 µL)	Wash Solution (30 µL)
Injection	Injection Solution (20 µL)	Injection Solution (20 µL)

Optimizing Plate Shaker for Sample Resuspension

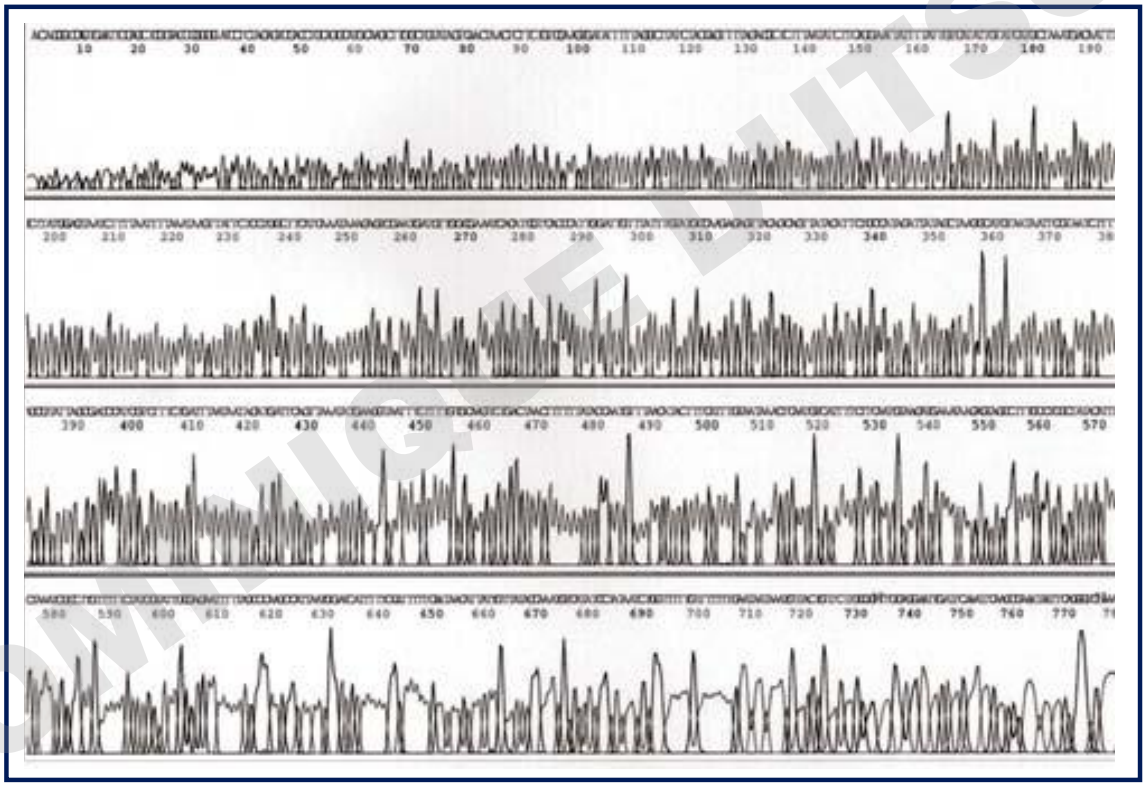
Perform the following optimization procedure for samples that are resuspended using a plate shaker.

1. Add 50 µL of a solution containing a dye such as bromphenol blue to each well of a SEQ_{96} plate. A previously used SEQ_{96} plate will work fine for this purpose.
2. Secure the plate on a microtiter plate shaker and begin shaking on the lowest setting.
3. Turn off the plate shaker after 30 seconds.
4. Blot the top of the plate using a white paper towel to reveal the presence of droplets.
5. If there is no evidence of droplets, repeat the procedure at the next highest shaker speed.
6. Continue increasing the shaker speed one setting at a time until droplets are found in order to determine the highest setting at which no drops are observed. This setting is the optimal speed to use to resuspend samples.

Product Performance

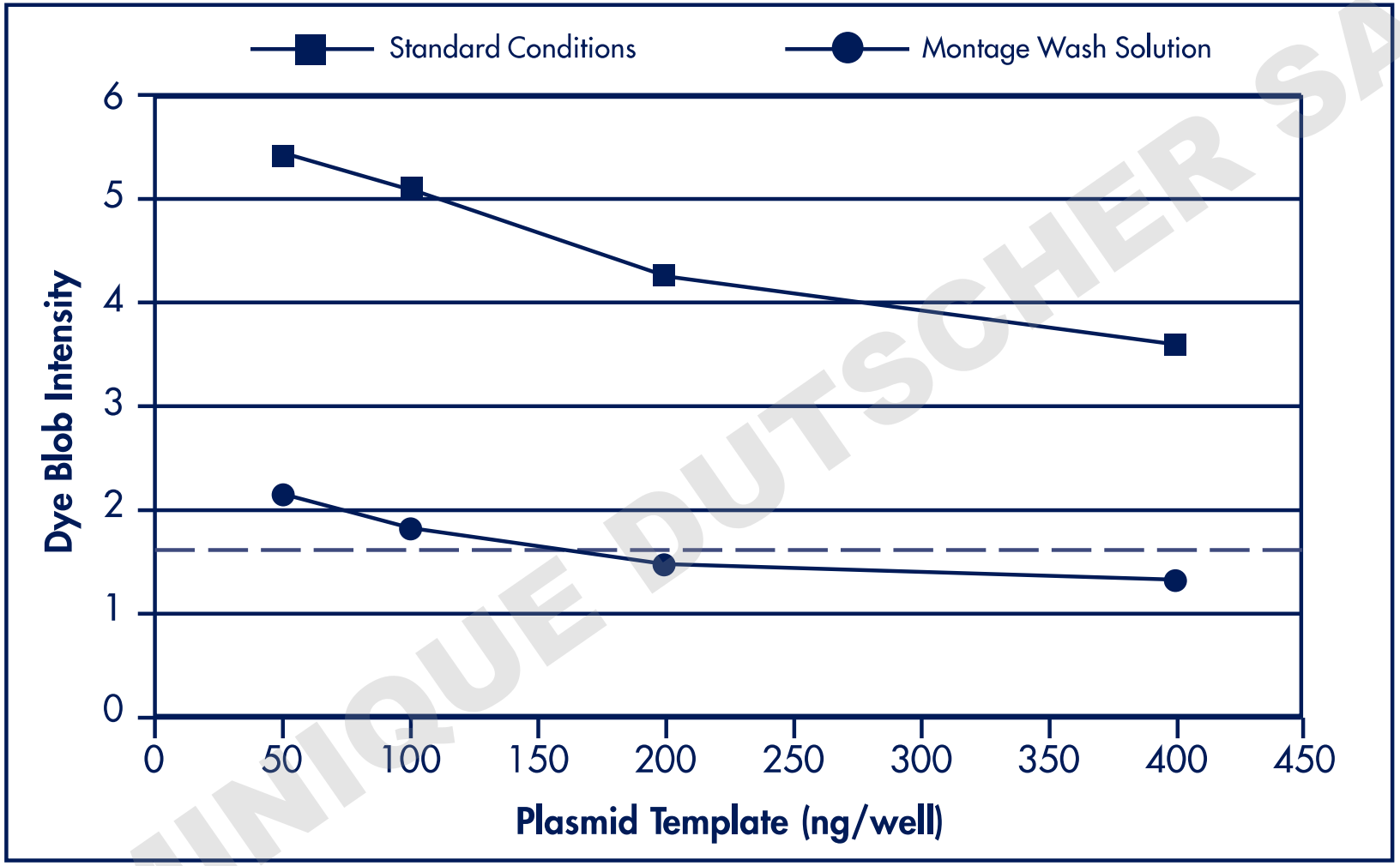
This section demonstrates typical results using the Montage SEQ_{96} Sequencing Reaction Cleanup Kit.

Figure 1. Electropherogram Showing Results Using Montage SEQ_{96} Kit



Plasmid DNA (200 ng), purified using a Montage Plasmid kit, was sequenced in a 1/8x BigDye Terminator v3.1 reaction. After purification using the Montage SEQ_{96} kit, the samples were analyzed on an ABI Prism 3700 capillary sequencer. This electropherogram is typical of the high quality reads achieved using this cleanup method (Phred 20 = 786).

Figure 2. Montage Wash Solution for Dye Blob Reduction



The magnitude of dye blobs is reduced by the Montage Sequencing Wash Solution. Comparison of 1/4x BDT v3.1 sequence reactions purified in the presence (circles) and absence (squares) of Montage Wash Solution. Significant reduction of dye blob intensity below threshold levels (dashed line) is achieved by using the Montage Sequencing Wash Solution for dilution and rinse. Sequence reads will typically start approximately 10–15 bases from the primer when the Sequencing Wash Solution is employed.

Troubleshooting

This section outlines how to troubleshoot possible problems encountered when using the Montage SEQ_{96} Sequencing Reaction Cleanup Kit.

Problem	Possible Causes	Suggestions
Low Signal	Dirty template/Low concentration of template added to sequencing reaction	Check template purity and concentration via gel electrophoresis. Use Montage Plasmid or PCR kit for optimal quality template.
	Inadequate resuspension of clean sequencing products from the SEQ plate	Increase the number of pipetting cycles or resuspend products on a plate shaker system after following the procedure in the "Optimizing Plate Shaker for Sample Resuspension" section.
Short or poor quality reads	Incorrect primer or template amount	Adjust primer and template concentrations: 5 pmol of primer and 150 to 400 ng of plasmid template.
	Over-drying the plate during filtration	Decrease vacuum duration. Do not allow the plate to remain under vacuum for longer than 1 minute once the solution is removed from the wells.

Troubleshooting, continued

Problem	Possible Causes	Suggestions
Tailing G or C peaks	Over-drying of the plate	Adjust filtration times so that the plate will not remain dry for more than 1 minute.
	Degradation of samples after purification	Inject samples immediately following purification.
Dye blobs	Incomplete removal of solution from wells	Increase vacuum duration. Do not allow the plate to remain under vacuum for longer than 1 minute once the solution is removed from the wells
	Inadequate consumption of dye terminators	Reduce amount of BDT used in sequence reaction (*i.e.*, reduce reaction mixture from 1/4x to 1/8x; see Table 1).
		Increase the amount of template.
		Increase the number of sequencing cycles.
		Use 30 µL of Montage Wash Solution (Millipore Cat. No. LSKS BW5 00) in Steps 3 and 10. See Table 3 for more information.

Ordering Information

This section lists catalogue numbers for the Montage SEQ_{96} Sequencing Reaction Cleanup Kit and accessories. See “Technical Assistance” for information about contacting Millipore. You can also buy Millipore products on-line at www.millipore.com/purecommerce.

Product	Catalogue No.	Qty/Pk
Montage SEQ_{96} kit, 1 pack	LSKS 096 01	1
Montage SEQ_{96} kit, 4 pack	LSKS 096 04	4
Montage SEQ_{96} kit, 24 pack	LSKS 096 24	24
Accessories		
Vacuum manifold	SAVM38401 or MSVM LOW 00	1
Vacuum pressure pump, 115V/60 Hz	WP61 115 60	1
Vacuum pressure pump, 220V/50Hz	WP61 220 50	1
Vacuum pressure pump, 100V/50–60Hz	WP61 100 60	1
Tubing, for vacuum use; silicone, 3/16” I.D., 1.4 m	XX71 000 04	1
Vacuum filtering flask, 1L	XX10 047 05	1
Stopper, No. 8, perforated, silicone	XX10 047 08	1

Ordering Information, continued

Product	Catalogue No.	Qty/Pk
Millex-FG$_{50}$ filter	SLFG 050 10	10
Montage Wash Solution	LSKS BW5 00	1
Montage Injection Solution	LSKS IS5 00	1

Plasmid Template Preparation

Product	Catalogue No.	Qty/Pk
Montage Plasmid$_{96}$ Miniprep Kit	LSKP 096 01	1
Montage Plasmid$_{96}$ Miniprep Kit	LSKP 096 04	4
Montage Plasmid$_{96}$ Miniprep Kit	LSKP 096 24	24

PCR Purification

Product	Catalogue No.	Qty/Pk
Montage PCR$_{96}$ Kit	LSKC 096 01	1
Montage PCR$_{96}$ Kit	LSKC 096 04	4
Montage PCR$_{96}$ Kit	LSKC 096 24	24
Montage PCR$_{\mu 96}$ Plates	LSKM PCR 10	10
Montage PCR$_{\mu 96}$ Plates	LSKM PCR 50	50

Ordering Information, continued

Sequencing reaction cleanup and PCR product purification in the 384 well format

Product	Catalogue No.	Qty/Pk
Montage SEQ_{384} plates	S384 SEQ 10	10
Montage SEQ_{384} plates	S384 SEQ 50	50
Montage PCR_{384} plates	S384 PCR 10	10
Montage PCR_{384} plates	S384 PCR 10	50

Technical Assistance

For more information, contact the Millipore office nearest you. In the U.S., call **1-800-MILLIPORE** (1-800-645-5476). Outside the U.S., see your Millipore catalogue for the phone number of the office nearest you or go to our web site at www.millipore.com/offices for up-to-date worldwide contact information. You can also visit the tech service page on our web site at www.millipore.com/techservice. For specific information about automated processes using Millipore products, go to www.millipore.com/automation.

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