User Guide

Montage® SEQ$_{96}$ Sequencing Wash Solution

For Removing Dye Blobs from Dye Terminator Sequencing Reactions

LSKSBW500

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures.

Introduction

The Montage® SEQ$_{96}$ Sequencing Reaction Cleanup Kit (LSKS09624) is designed for the removal of unincorporated dye terminators and residual salts from sequencing reactions.

For use in reactions that utilize high concentrations of sequencing chemistries (1/2x and 1/4x), we have developed a novel Sequencing Wash Solution, designed to remove dye blobs. This solution is intended to be a complement to the Injection Solution that is supplied with the Montage® SEQ$_{96}$ Cleanup Kit. (The Injection Solution is also available separately, as LSKSIS500.)

The following directions are recommended for all versions of BigDye® and DYEnamic® ET Terminator chemistries.

Materials Provided

Montage® SEQ$_{96}$ Sequencing Wash Solution (LSKSBW500). One bottle containing 500 mL of sequencing wash solution. Store at room temperature.

Warnings and Precautions

Use personal protective equipment as required. Avoid skin contact or ingestion of all reagents and chemicals used in this protocol.

Storage and Stability

Store at room temperature; performance guaranteed for 1 year from date of receipt when solution is stored properly.

Directions for Use

1. Set up the sequencing reactions in a thermal cycling plate and amplify using an appropriate thermal cycling program.
2. After thermal cycling, add 30 µL of the Sequencing Wash Solution (LSKSBW500) to each of the sequencing reactions in the 96-well thermal cycling plate and mix gently.
3. Transfer all of the mixture to the Montage® SEQ$_{96}$ plate.
4. Place the plate on the vacuum manifold (SAVM38401) and apply vacuum for 3-4 minutes (until no fluid remains in the wells), with the vacuum pump set at 23–25 inHg. Continue the vacuum for about 15 seconds after the last well is empty.
5. Shut off the vacuum and remove the plate from the manifold. Blot the excess fluid from the bottom of the plate by pressing the plate briefly to a stack of paper towels.
6. Return the Montage® SEQ$_{96}$ plate to the vacuum manifold. Add 30 µL of the Sequencing Wash Solution.
7. Apply vacuum for 3-5 minutes (until no fluid remains in the wells), with the vacuum pump set at 23–25 inHg. Continue the vacuum for about 15 seconds after the last well is empty.
8. Shut off the vacuum and remove the plate from the manifold. Blot the excess fluid from the bottom of the plate, by pressing the plate briefly to a stack of paper towels.

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9. Add 25 µL of the Injection Solution to each of the purified sequencing reactions in the Montage® SEQ_{96} plate. Resuspend the DNA by pipetting up and down 15-20 times, if using an automated liquid handler. If desired, the DNA can be resuspended by shaking for 10 minutes at an appropriate speed on a micro-plate shaker. For example, use setting "8" on the 4-position shaker made by LabLine.
10. Transfer the purified sequencing products to an appropriate plate for sequencing.

To Prevent Evaporation When Injecting Samples into the ABI Prism® 3100

1. Resuspend the sequencing reactions in 20 µL of Injection Solution by pipetting or shaking. Transfer the samples to an appropriate plate for sequencing.
2. After the samples have been transferred, add 15 µL of HiDi formamide (from ABI) to each well. This will prevent evaporation.
3. Inject into the sequencer.

Sequencing Reaction Composition

Component	**1x**	**1/2x**	**1/2x**	**1/4x**	**1/4x**	**1/8x**
Template[1] Plasmid (50 ng/µL)[2] PCR (5 fmol/µL)	2.0 µL	2.0 µL	2.0 µL	2.0 µL	2.0 µL	2.0 µL
2.5x Buffer	0.0 µL	4.0 µL	0.0 µL	2.0 µL	0.0 µL	1.0 µL
Primer (5.0 pmol/µL)	1.0 µL	1.0 µL	1.0 µL	1.0 µL	1.0 µL	1.0 µL
BDT Premix	8.0 µL	4.0 µL	4.0 µL	2.0 µL	2.0 µL	1.0 µL
Milli-Q® Water[3]	9.0 µL	9.0 µL	3.0 µL	3.0 µL	0.0 µL	0.0 µL
Total	**20.0 µL**	**20 µL**	**10.0 µL**	**10.0 µL**	**5.0 µL**	**5.0 µL**

*2.5x Buffer: 200 mM Tris-HCl, pH 9.0, 5 mM $MgCl_2$

[1] Template quality has the most dramatic effect on sequencing quality. For optimal results, we recommend that plasmid, BAC and PCR templates be prepared with Montage kits.

[2] Efficacy has been demonstrated at template amounts from 80-500 ng per reaction. The protocol has been optimized for 100 ng of template.

[3] BigDye mix, primer, 2.5x buffer and water are typically mixed together to make a sequencing "cocktail." An appropriate volume of cocktail is then dispensed into each well.

Recommended Injection Conditions

ABI 3 100	**3100-Avant**	**3730**	**730 xl**	**3700**	**MegaBACE®**
1.5 kV	1.0 kV	Follow AB default injection conditions		2.0 kV	2.0 kV
20 sec	15 sec			15-30 sec	30-40 sec

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