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Montage PCR₉₆ Cleanup Kit User Guide

GENOMICS

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P36316 Rev. —, 11/00

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Introduction

The Montage™ PCR₉₆ Cleanup Kit provides the materials and the protocol required to prepare purified samples that can be used for a variety of applications. Using proprietary membrane technology, Millipore has developed a system for purifying PCR products that eliminates the need for bind/elute methods and centrifugation. This system also significantly reduces sample processing time. MultiScreen™₉₆ PCR plates are single-use 96-well filter plates developed for the purification of PCR products in the 0.05 to 0.3 mL volume range. MultiScreen₉₆ PCR plates are designed for vacuum filtration with the MultiScreen vacuum manifold.

Automation with MultiScreen₉₆ PCR Plates

The Montage PCR₉₆ Cleanup Kit is easily automatable, with all sample manipulations occurring on the surface of the membrane and no washing steps or filtrate collection required. Protocol optimization (sample process time and recovery) with any one liquid pipetting instrument is generally a function of the mixing step. A successfully automated protocol requires an instrument capable of providing a vigorous agitation of the reconstituted sample, in a 5–10 minute mix step.

NOTE: If the liquid pipetter in use is not capable of performing this mix step (up and down pipetting cycles), a plate shaker may be required for this step.

Another important criteria for an optimized automated protocol is the volumetric recovery of the sample, which can be maximized by ensuring that the instrument deck is level, and that the tips are programmed to aspirate the sample as close to the membrane as possible. MultiScreen₉₆ PCR plates are compatible with a wide range of liquid handling instruments. However, in some cases, a semi-automated protocol (using a plate shaker for the sample agitation step) results in optimal performance (recovery and speed). Recommendations can be made for an automated or semi-automated protocol, as outlined in the following table.

Automated vs. Semi-Automated Protocol

Liquid Handler	Approximate Protocol Time (minutes per single 96-well plate)	
	Fully-automated*	Semi-Automated (with plate shaker)**
Beckman Multimek™ (96 tips) <i>Can be automated; Technical Note #TN1546EN00*</i>	12 minutes	15-20 minutes
Beckman FX (96 tips) <i>Can be fully automated; contact Millipore Technical Service for support</i>	12 minutes	15-20 minutes
Beckman Biomek® 2000 (8 tips) <i>Can be automated; Technical Note in progress; contact Millipore Technical Service for support</i>		Under evaluation
Tecan Genesis™ (8 tips) <i>Can be automated; contact Millipore Technical Service for support</i>		Under evaluation
TomTec Quadra™ (96 tips; 6-position deck) <i>Can be fully automated; Technical Note #TN1545EN00</i>	25 minutes	20 minutes

Automated vs. Semi-Automated Protocol, continued

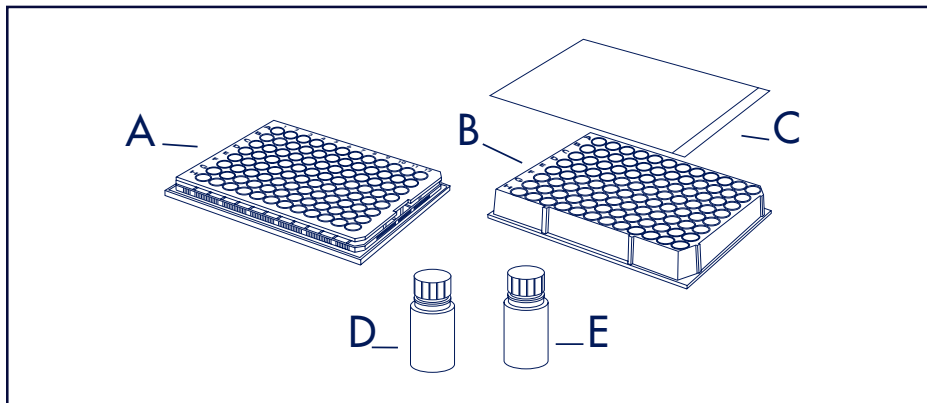
Approximate Protocol Time (minutes per single 96-well plate)

Liquid Handler	Fully-automated*	Semi-Automated (with plate shaker)**
MWG		Under evaluation
<i>Contact Millipore Technical Service for support.</i>		
Qiagen BioRobot®		
· 1-tip	Under evaluation	
· 2-tip	· 55-65 min	
· 8-tip	Under evaluation	
<i>Can be automated. Contact Millipore Technical Service for support.</i>		
Packard® Multiprobe		
· 4 tip		Under evaluation
· 8 tip		Under evaluation
<i>Can be semi-automated; plate shaker recommended for sample mixing step.</i>		
Robbins Hydra® (96 tips)		Under evaluation
<i>Can be semi-automated; plate shaker recommended for sample mixing step.</i>		

*The manifold can be put on the instrument deck if a hole is cut in the table or a 4-position table is used instead of the typical 6-position table.

**Requires manual move of plate from vacuum manifold and instrument deck to plate shaker, and plate shaker back to vacuum manifold.

Kit Components



Letter	Part	Function
A	MultiScreen ₉₆ PCR plate	Purification of PCR products
B	V-bottom storage plate	Storage of purified samples
C	Sealing tape	Sealing of V-bottom plate
D	Nuclease-free water	Washing of PCR products and resuspension/ storage of purified samples
E	Tris buffer	Resuspension/storage of purified samples

Additional Equipment Required

In order to use the Montage PCR₉₆ Cleanup Kit, you will need:

- Vacuum manifold with underdrain support grid (Millipore Cat. No. MAVM 096 0R, or equivalent).
- Portable vacuum pump or uniform vacuum source (Millipore Cat. No. XX55 000 00, or equivalent).

NOTE: Millipore recommends that you use a portable vacuum pump for vacuum consistency. The pump enables you to maintain constant pressure and obtain reproducible results.

- Plate shaker or liquid handler capable of providing vigorous agitation of reconstituted samples.

Precautions

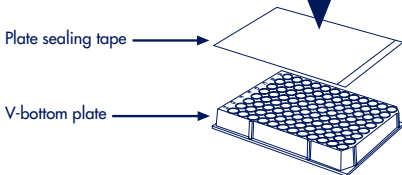
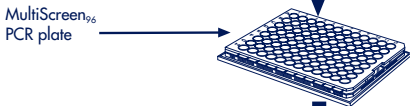
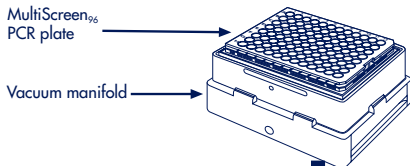
- MultiScreen plates are disposable, single-use-only devices.
- This kit is for research use only. Not for use in clinical applications.
- Operate at ambient temperature.
- Not for use in centrifugal mode.
- For use with vacuum only. Filtration time varies depending on volume added to the wells and the strength of the vacuum source. It is important that all wells are completely emptied of liquid before resuspending purified PCR products.
- MultiScreen₉₆ PCR plate consists of a polystyrene plate which is sealed to a polyethylene underdrain, forming 96 independent (individually-sealed) wells. **Do not separate the MultiScreen polystyrene plate from the underdrain. Separation will result in plate failure and well leakage.**
- **The vacuum manifold must have an underdrain support grid or the MultiScreen₉₆ PCR top plate will irreversibly separate from the underdrain upon vacuum application.**

Storage Conditions

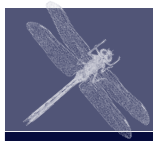
This kit should be stored at 15°C to 30°C.



Overview of Procedure for Purification of PCR Products



1. Load PCR samples into MultiScreen₉₆ PCR plate.
2. Place MultiScreen₉₆ PCR plate on top of vacuum manifold.
3. Apply vacuum for 5–10 minutes.
4. Resuspend samples.
(Mix vigorously.)
5. Transfer samples to V-bottom plate for storage.
Seal wells with sealing tape.



Purification Protocol for PCR Products

The protocol below outlines the steps necessary to load the PCR reactions into the MultiScreen₉₆ PCR plate, purify the samples using vacuum filtration, and reconstitute the samples using either a liquid handler or plate shaker.

1. Load PCR reactions into the MultiScreen₉₆ PCR plate (typically 100 μ L, up to 300 μ L of PCR sample per well). You do not need to use all 96 wells at the same time.
2. Place the MultiScreen₉₆ PCR plate on top of the MultiScreen manifold.
3. Apply vacuum at 24 inches Hg (812.7 millibar = 609.6 torr) for 5–10 minutes or until wells have emptied. Starting volumes larger than 100 μ L will take longer to empty. Allow 30 extra seconds under vacuum after the wells appear empty to be sure all liquid has filtered. The filters will appear shiny even after the wells are empty.

NOTE: If you determine the purity of the PCR products is not sufficient for a particular application, you can follow this step with a wash consisting of 100 μ L of the provided nuclease-free water.

Purification Protocol for PCR Products, continued

4. Reconstitute the samples with nuclease-free water or Tris buffer as follows:

When using a liquid handler: Leave the plate on the manifold (with vacuum off) and add water or buffer (100 μ L) to each well. Mix sample by pipetting up and down vigorously. (For example, the Beckman MultiMek 96 can be used to dissolve samples with 100 μ L of buffer by adjusting the tip height just above the membrane surface and pipetting 90 μ L up and down for 10 cycles at 50% speed setting.)

NOTE: Do not use a manual multichannel pipette to resuspend the samples. Manual pipetting for this step results in high well to well variability.

When using a plate shaker: Before using the plate shaker for the first time, perform the procedure outlined in the "Optimizing Plate Shaker for Sample Resuspension" section. Then proceed as follows: Remove the plate from the manifold, blot from underneath with paper towels, and add water or buffer (50-100 μ L) to each well with a liquid pipetter. Mix sample vigorously on a plate shaker for 5 minutes.

5. Retrieve purified PCR products from each well by pipetting and transferring to V-bottom plate for storage.

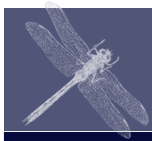
Optimizing Plate Shaker for Sample Resuspension

If you are using a plate shaker, perform the following optimization for resuspension to determine the correct speed setting for your plate shaker. In order to perform this procedure, you will need a used (or new) MultiScreen₉₆ PCR plate and buffer that has been dyed (using bromphenol blue or something similar) so that it will be easy to see. This procedure requires that you intentionally shake the buffer out of the wells.

1. Add the volume of buffer to each well that you intend to use to resuspend the purified PCR products (e.g., 50 μ L).
2. Secure the plate on a microtiter plate shaker and begin shaking on the lowest setting.
3. Turn off the plate shaker after about 30 seconds.
4. Examine between the wells on the top surface of the MultiScreen₉₆ PCR plate to detect the tiny droplets that will form if the shaking speed is too vigorous. (A white paper towel will also help show these tiny colored droplets if you blot the top of the plate evenly.)
5. Proceed to the next highest shaker setting if there is no evidence of droplets, shake for 30 seconds, then check for droplets.

Optimizing Plate Shaker for Sample Resuspension, *continued*

6. Continue increasing the shaker speed one setting at a time until you detect droplets on the top surface of the plate.
7. Reduce the shaker speed by one setting and record the speed setting. Subsequently, you may use this setting for resuspension of your purified samples.



Product Performance

This section outlines the overall recovery of PCR fragments using the Montage PCR₉₆ Cleanup Kit.

137 bp	301 bp	500 bp	657 bp	1,159 bp
70.9 ±14.1%	77.1 ±9.5%	89.7 ±3.8%	88.3 ±11.1%	89.9 ±6.4%
CV = 19.9%	CV = 12.3%	CV = 4.3%	CV = 12.6%	CV = 7.1%

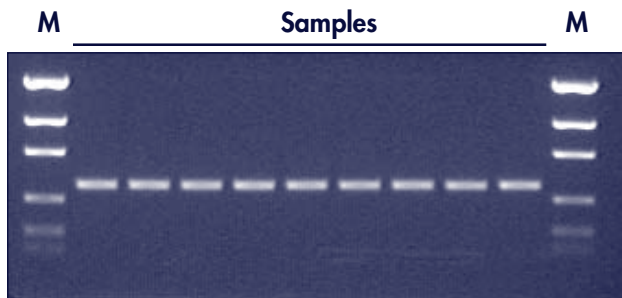


Figure 1. Agarose gel showing reproducibility of recovering a 500bp PCR product with Montage PCR₉₆ Cleanup Kit.



Troubleshooting

This section outlines how to troubleshoot problems you may encounter when using the Montage PCR₉₆ Cleanup Kit.

Problem	Possible Causes	Suggestions
Low yields shaker	Insufficient mixing	Optimize pipetting station/plate
	PCR product is below 137 bp	Use MultiScreen FB plates and protocol
	PCR product has dried on plate	Process immediately after filtering or rehydrate and store
	PCR product recovered in <50 μ L	Increase volume to 50 μ L minimum
Sample contamination	Sample not filtered to dryness	Vacuum until wells have emptied.
Plates separating	No underdrain support grid	Use Millipore manifold or equivalent with support grid.
Wells not filtering	Airlock due to bubble in the well	Agitate the samples until bubble surfaces.



Specifications for MultiScreen₉₆ PCR Plate

Maximum operating sample capacity: 0.3 mL

Hold-up volume membrane and support: 4 μ L

Maximum vacuum pressure: 25 inches Hg/635 Torr

Active membrane area of well: 0.2 cm²

Dimensions of assembled plate:

Plate length: 123.4 mm

Plate width: 82.7 mm

Plate depth: 14.6 mm without cover

16.5 mm with cover

Materials of construction:

Plate: Polystyrene

Underdrain Support: Polyethylene

Membrane: Proprietary



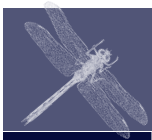
Ordering Information

This section lists catalogue numbers for the Montage PCR₉₆ Cleanup Kit. 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 PCR ₉₆ Cleanup Kits: for 96 samples	LSKC 096 01	1/pk
Montage PCR ₉₆ Cleanup Kits: for 4 × 96 samples	LSKC 096 04	4/pk
Montage PCR ₉₆ Cleanup Kits: for 24 × 96 samples	LSKC 096 24	24/pk
MultiScreen ₉₆ PCR Plates	MANU 030 10	10/pk
MultiScreen ₉₆ PCR Plates	MANU 030 50	50/pk
Nuclease-free water, 500 mL	LSKN F05 00	1/pk
Tris buffer, 500 mL	LSKC TB5 00	1/pk
V-bottom plates	LSKV BP1 00	100/pk
Adhesive plate sealing tape	LSKA ST1 00	100/pk
Millipore filtration manifold	MAVM 096 0R	1/pk

Ordering Information, continued

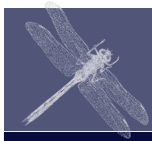
Product	Catalogue No.	Qty/Pk
Vacuum/pressure pump, 115V/60Hz	XX55 000 00	1/pk
Vacuum/pressure pump, 220V/50Hz	XX55 220 00	1/pk
Vacuum/pressure pump, 100V/50 or 60Hz	XX55 100 00	1/pk



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 laboratory catalogue for the phone number of the office nearest you. You can reach us by e-mail at tech_service@millipore.com or visit our web site (www.millipore.com).

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