Millipore®

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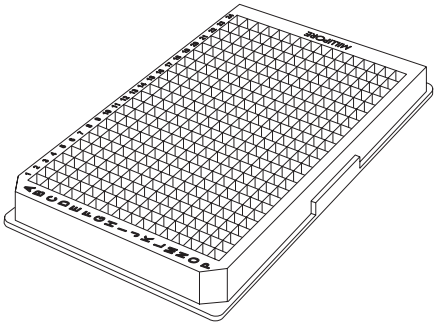
User Guide

MultiScreen®$_{384}$-SEQ Plates



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S384SEQ10
S384SEQ50

Introduction

MultiScreen®$_{384}$-SEQ plates are intended for the removal of salts and unincorporated dye terminators from sequencing reactions prior to DNA sequencing. The protocol is fast, easy to perform, and has proven effective in removing both major types of commercially available energy transfer dyes (BigDye® Terminators and DYEnamic® ET Terminators). Filtering the wells of the plate to dryness purifies the sequencing products by washing salts and unincorporated dye terminators to waste. The purified sequencing products are then resuspended from the membrane surface and are ready for injection into a capillary or slab gel type DNA Sequencer.

Protocol Guidelines

- Complete filtration is required for sample purity. The filters appear shiny even after they are dry. Continue filtration for 15–30 seconds after the last well appears empty.
- Filtration time will vary depending on the type of sequencing reactions being purified.
- Do not exceed the recommended vacuum pressure (25 inHg).

Procedure

The filtration times stated in the procedure below were developed using 1/8th BigDye® reactions.

1. After thermal cycling, adjust sequencing reaction volume to 20 µL with 0.3 mM EDTA, pH 8.0 made in Milli-Q® grade water in the 96-well or 384-well thermal cycling plate. Mix gently by pipetting up and down 3–5 times.
2. Transfer all of the sequencing reaction from the thermal cycling plate to the MultiScreen®$_{384}$-SEQ plate.
3. Place the MultiScreen®$_{384}$-SEQ plate on the vacuum manifold. Set the vacuum pump to 23–25 inHg and apply the vacuum for 3–4 minutes, or until the wells are completely empty. Keep applying the vacuum for an additional 15–30 seconds after the last well is empty.
4. Shut off the vacuum source and remove the MultiScreen®$_{384}$-SEQ plate from the manifold.
5. Blot the excess liquid from the bottom of the MultiScreen®$_{384}$-SEQ plate by briefly pressing the plate on an absorbent material such as paper towels.
6. Place the MultiScreen®$_{384}$-SEQ plate back on the vacuum manifold. Add 25 µL of 0.3 mM EDTA, pH 8.0 to every well of the MultiScreen®$_{384}$-SEQ plate.
7. Apply vacuum at 23–25 inHg 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.
8. Shut off the vacuum source and remove the MultiScreen®$_{384}$-SEQ plate from the manifold.
9. Blot the excess liquid from the bottom of the plate by briefly pressing the plate on an absorbent material such as paper towels.
10. To resuspend the sequencing products, add 20 µL of 0.3 mM EDTA, pH 8.0 and pipette up and down for at least 20 cycles with a liquid handler.
11. Transfer the purified sequencing products to an appropriate plate for electrokinetic injection

    To inject samples onto the sequencer using a high concentration formamide buffer (>20% Formamide).

    Resuspend the samples in Milli-Q® grade water and transfer them to an appropriate plate for loading onto the sequencer. To accelerate the evaporation, dry the sample using Speedvac™ or similar device, and then resuspend the samples in the formamide buffer. The samples are now ready for loading onto a gel or for electrokinetic injection.
12. Samples should be injected at 2 kV for 15–30 seconds into the ABI Prism® 3700 sequencer or at 2 kV for 30–40 seconds onto the MegaBace® 1000 sequencer. For the 3730, 3730XL, 3100 and 3100 Avant instruments, use the injection parameters supplied by ABI.

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Specifications

SBS/ANSI compliant	
Maximum well volume	130 µL
Working well volume	100 µL
Recommended vacuum pressure	25 inHg
Distance from bottom of the plate to membrane surface	3 mm
Materials of Construction	
Plastic housing	Clear polystyrene
Membrane	Proprietary

Product Ordering

Purchase products online at SigmaAldrich.com.

Description	Qty.	Catalogue No.
MultiScreen®$_{384}$-SEQ plate	10/pk	S384SEQ10
MultiScreen®$_{384}$-SEQ plate	50/pk	S384SEQ50
MultiScreen®$_{384}$-PCR plate	10/pk	S384PCR10
MultiScreen®$_{384}$-PCR plate	50/pk	S384PCR50
Vacuum Manifold for 384 well plate, includes base, tubing, gauges, and flow control.	1/pk	SAVM38401
Tubing, for vacuum use; silicone, 3/16 in. I.D., 1.4 m	1/pk	XX7100004
Vacuum filtering flask, 1L	1/pk	XX1014705
Stopper, No. 8, perforated, silicone	1/pk	XX1014708
Millex®-FG$_{50}$ filter device	10/pk	SLFG05010
Chemical Duty Pumps		
115 V, 60 Hz	1/pk	WP6111560
230 V, 50 Hz	1/pk	WP6122050
100 V, 50/60 Hz	1/pk	WP6110060

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