Technical Note

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Title: Guidelines For Centrifugal Filtration with MultiScreen® Plates

*****PLEASE NOTE*****

Many of the items listed below are no longer available however Millipore does offer excellent substitutes. Please call technical support for detailed information on any product differences.

Materials Used	***Materials Currently Available***
MHVBN4510	*MSHVN4B10**
MAHVN4510	*MSHVN4510**
MAFBN0B10	*MSFBN6B10**
MAFCN0B10	*MSFCN6B10**
MAPHN0B10	*MSPHN6B10**
MAVMN0510	*No longer available*
MADPN0B10	*No longer available*
MAVM09601	*MSVMHTS00*

** Centrifuge alignment frames no longer needed with replacement plates*

Introduction

The MultiScreen assay system is based on a 96-well filter plate containing different types of microporous media, instead of a simple plastic bottom. While every step of an assay, including incubations, washes and detection steps can be carried out within a single plate, it is necessary to remove wash fluid and other reagents after the consecutive assay steps. This procedure is usually carried out using the MultiScreen or a robotic vacuum manifold because it is much faster. There are, however, situations when use of a swinging bucket centrifuge with rotors for microplates is advised for collecting filtrates:

- The use of 96 well 'mini-columns' containing soft gel separations media such as Sephacryl®-500-HR for DNA purification after PCR (Wang, et al. 1995) or Sephadex® G-25 or G-50 for desalting applications (Millipore Technical Note, TN050). With these "mini-column" applications in MultiScreen plates there is a need to use a centrifuge instead of the vacuum manifold. This is because of the need to assure the proper packing of the columns. Optimal packing is not achievable using a vacuum manifold and may result in channeling and cracking of the bed.

- The quantitative collection of filtrates containing high levels of solvents such as alcohols (>40%) or any surfactants and detergents such as Tween and sodium dodecyl

sulfate (SDS). Vacuum filtration of the these fluids may result in mis-transfer from the plate into the receiver plate. Results included in this technical note demonstrate filtrate collection of these solvents.

- Researchers interested in using the MultiScreen plates containing VMWP membrane, due to the small 0.05µm pore size of this filter, need to use centrifugal filtration to effectively remove fluids from the wells.

- This method can be used to recover results from an experiment where the filter has been plugged and vacuum is not emptying the wells.

Low speed centrifuges (1,000 x g) designed for spinning 96-well plates can be easily used for these applications. The purpose of this Technical Note is to report recommended MultiScreen centrifugation procedures compared to vacuum filtration showing typical results obtained for sample and filtrate recoveries. In addition, filtrate collection results for low surface tension liquids are also reported.

Materials and Methods

Materials:

- MultiScreen -HV, Opaque Plates. Cat. No. MHVBN4510
- MultiScreen -HV, Clear Plates. Cat. No. MAHVN4510
- MultiScreen -FB, Opaque Plates. Cat. No. MAFBN0B10
- MultiScreen -FC, Opaque Plates. Cat No. MAFCN0B10
- MultiScreen -PH, Opaque Plates. Cat. No. MAPHN0B10
- MultiScreen -VM, Clear Plates. Cat. No. MAVMN0510
- MultiScreen -DP, Opaque Plates. Cat. No. MADPN0B10
- PBS with 0.01% Sodium Azide
- 0.0025% Bromophenol Blue (BB) in PBS
- 1 mg/ml Bovine Serum Albumin (BSA) in PBS
- Total Protein Assay BCA Reagents A + B (Pierce #23223, #23224)
- 8 Channel Pipetter
- Molecular Devices THERMOmax™ Reader (O.D. 562 nm) with SOFTmax® PRO data analysis Software (v1.2.0)
- 96 well flat bottom collection plate
- Vacuum Manifold (Millipore #MAVM09601)
- Centrifuge alignment frame (Cat. No. MACF09604 for aqueous solutions or MACF096S4 for solvents)

Unless specified, all reagents were purchased from Sigma Chemical Company.

CV% Liquid Transfer Test and Dead Volume Procedure:

This procedure was used to test the accuracy as measured by calculating the Coefficient of Variation (% CV= s.d. ÷ mean x 100 for all 96 wells) of liquid transfer across the membranes on the MS plates and to determine the amount of residual liquid left at the end of draining a plate.

1. Weigh dry plates and record.
2. Add 100 ul 0.0025% BB in PBS to all wells using 8 Channel Pipetter.
3. Centrifuge into 96 well flat bottom collection plate.
4. Add 100 ul PBS to all wells.
5. Centrifuge into same 96 well flat bottom collection plate.
6. Weigh wet plates immediately and record.
7. Use Molecular Devices Microplate Reader to get O.D. @562 nm and calculate the plate statistics.
8. Repeat steps 1-8 for same plates using Vacuum Manifold instead of centrifuge.

BSA Recovery and Dead Volume Procedure:

This procedure was used to test the % of protein (product) recovered in the filtrate collection plates after the experiment. The dead volume calculation is also performed for the assorted MS plates with centrifugation and vacuum filtration methods compared.

1. Block rows 10, 11, 12 on plates with tape.
2. Weigh plates and record.
3. Add 100 ul 1 mg/ml BSA in PBS to rows 1-8.
4. Add 100 ul PBS.
5. Spin in centrifuge into 96 well flat bottom collection plate.
6. Weigh plates and record.
7. Set up a BSA serial dilution in the empty wells of the 96 well flat bottom collection plate to use as a standard curve.
8. Add 150 ul of mixed Pierce Protein Assay Reagents A and B.
9. Incubate at 37 ºC for 30 minutes.
10. Use Molecular Devices Microplate Reader to obtain and analyze results.

Rotor/Centrifuge Compatibility:

The following centrifuge rotors and carrier racks with 96-well plate carriers capable of holding two plates have been tested for compatibility with the MultiScreen system:

Beckman JS 4.2, JS 3.0, and JS 4.3
IEC 5783
IEC 49852
Jouan CR4-22, M4 rotor: carrier #1174168
Sorvall PN11065
Beckman GH3.7 rotors
Beckman TH-4 rotor

Microtiter Plates for Collecting Filtrate:
Costar plates (Costar # 9017, Fisher # 504-012-76) were used at the time of testing however any SBS compliant 96 well solid bottom plate can be used. Millipore offers these plates under catalogue number MSCPNPS00. These plates will withstand 1000 x g, but many microtiter plates will fracture at 1500 to 2000 x g.

The use of the MultiScreen Centrifuge Alignment Frame (Catalog #MACF09604 for aqueous solutions or MACF096S4 for solvents) will allow the MultiScreen filtration plate to "lock" onto any standard 96-well plate, **including V-bottom plates**, during centrifugation. This frame ensures plate alignment and quantitative filtration collection regardless of the rotor.

Reported results were achieved using Beckman centrifuges (Beckman J2-HC, Beckman TJ-6). The rotors used were a Beckman JS4.3 with Microplus carriers (2000 rpms) and Beckman TH-4 with Microtitre plate carriers (2300 rpms), respectively. All samples were spun at approximately 910 x g for 5 minutes.

Results and Discussion

Liquid Transfer and Dead Volume Test:

Plate Type	Dead Volume / well (ul) **Centrifuge**	Dead Volume / well (ul) **Vacuum**	Liquid Transfer CV% **Centrifuge**	Liquid Transfer CV% **Vacuum**
FB	20.8	20.3	1.797	0.870
HV OPAQUE	3.2	3.8	1.259	0.823
FC	12.6	11.4	0.739	0.894
PH	14.0	8.3	1.654	0.877
HV CLEAR	3.5	2.9	0.615	1.594
VM	3.5	5.2	0.884	0.509

BSA Recovery and Dead Volume:

Plate Type	Dead Volume / well (ul) **Centrifuge**	Dead Volume / well (ul) **Vacuum**	% Volume Recovery **Centrifuge**	% Volume Recovery **Vacuum**	% BSA Recovery **Centrifuge**	% BSA Recovery **Vacuum**
FC	12.7	15.9	87.3	84.1	81.2	75.4
FB	22.8	21.4	77.2	78.6	67.1	68.2
HV CLEAR	3.4	7.6	96.6	92.4	92.1	88.3
HV OPAQUE	3.8	8.7	96.2	91.3	93.4	88.6

VM	3.6	2.9	96.4	97.1	42.7	46.8
DP	3.6	6.5	96.4	93.5	94.6	87.2

The results show no significant differences between centrifugation and vacuum filtration. The larger dead volume MS plates were typically the glass fiber and paper stuffed wells, with the increased dead volumes consistent with the thickness of the filter material. The glass fiber type B is the thickest material followed by the type C glass and phosphocellulose paper. The amount of filtrate passed through the membrane, as tested by the liquid transfer test, showed little variation from one method to the other. The BSA recovery procedure tests for product recovery and also showed little difference between methods. The dead volume, the amount of solution left in the MultiScreen plate after filtration, was also fairly constant when comparing centrifugation and vacuum filtration.

Low Surface Tension Liquid Recovery

Complete recovery of low surface tension liquids in the filtrate is difficult with vacuum filtration. Note that a non-surfactant wash following surfactant-containing reagents restores the quantitative filtrate collection. However, it is possible to use a centrifuge for quantitative filtrate collection of liquid samples containing surfactant or high alcohol concentrations. Recovery was tested in 70% EtOH, 1% SDS in PBS, and 0.05% Tween 20 in PBS. All of the testing was done with 0.0025% Bromophenol Blue except for the 0.05% Tween 20 which contains 0.00125% BB.

Plate	Liquid Used	CV%
HV Opaque	70% EtOH	2.435
IP	70% EtOH	1.209
HV Clear	1% SDS	1.281
HV Clear	.05% Tween 20	1.701

Summary

The procedures and results reported here demonstrates both the utility and recommended applications for using the centrifuge to achieve the desired separations with the MultiScreen plates. Although using the centrifuge takes more time to achieve efficient liquid transfer, it is recommended for those applications requiring packing of “mini-columns” of soft gel media and for collecting low surface tension liquids. By adding centrifugal separation to commonly used MultiScreen procedures, these two applications are now relatively convenient to perform.

References

1. Wang, K. Gan, L., Boysen, C., and Hood, L. (1995). A Microtiter Plate-Based High throughput DNA Purification Method, Analytical Biochemistry. **226:** 85-90.

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