

User Guide

MultiScreen® Permeability Filter Plate Assembly

for Parallel Artificial Membrane Permeability Assay (PAMPA)

Cat. No. MPC4NTR10

Introduction

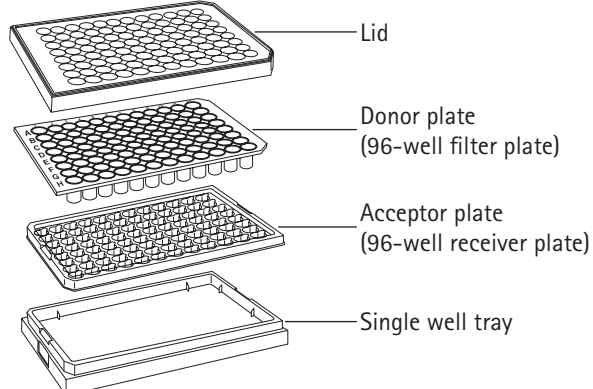
The MultiScreen® Permeability Filter Plate Assembly is a 96-well disposable device designed to support high throughput non-cell-based assays, such as the assessment of permeability for drug compounds. An artificial membrane, typically constructed of hexane/hexadecane, is created in the pores of the track-etched polycarbonate filter in the 96-well filter plate before the assay is performed.

After creation of the artificial membrane, the wells of the filter plate (known as the donor plate) are filled with the drug compounds to be tested. The filled donor plate is then placed upon a 96-well receiver plate (known as the acceptor plate) filled with buffer. This acceptor plate can be either the plate provided with this assembly or an equivalent, such as the polytetrafluoroethylene (PTFE) acceptor plate (cat. no. MSSACCEPTOR). The donor and acceptor plates are incubated together, typically for 5 to 7 hours, then the donor plate is removed from the acceptor plate.

Once separated, samples are taken from both the donor and acceptor wells (192 samples total) and assayed to determine how much compound has passed through the artificial membrane. Samples can be assayed by Liquid Chromatography/Mass Spectrometry (LC/MS) analysis or transferred to UV-compatible 96-well plates and read immediately with a UV/Vis spectrophotometer. The integrity of the artificial membrane layer in the donor plate can be confirmed using electrical resistance.

This permeability assay simulates transcellular drug absorbance rates.

Materials Supplied



Materials Required

In addition to the MultiScreen® Permeability Filter Plate Assembly, the user must supply the following materials and equipment:

- Drug compound samples to be evaluated, in concentrated or pre-prepared dilutions
- Aqueous transport buffer
- Artificial membrane solution and solvent
- UV/Vis spectroscopic microplate reader, HPLC/UV, or LC/MS/MS
- Optional - MultiScreen®_{HTS} 96-well UV-compatible Collection Plate (cat. no. MSCPNUV40)
- Optional - MultiScreen® Acceptor Plate (cat. no. MSSACCEPTOR)
- Optional - MultiScreen® 96-well Deep Well Collection Plate (cat. no. MDCPN2M50)

Usage Guidelines

- For research use only
- Single use only
- Plates are designed to support aqueous transport assays. Solvent systems should be tested prior to running samples.
- See references 1–7 for additional details on running PAMPA assays.
- The single well tray is provided to protect the filters on the bottom of the donor plate wells during artificial membrane formation and sample loading.

Permeability Assay Procedure

Guidelines for Setting Up the Assay

- Monitor your multichannel pipette to ensure that all tips are drawing and dispensing volumes accurately.
- The 96-well Deep Well Collection Plate (cat. no. MDCPN2M50) is useful for preparing drug samples.
- Consider testing permeability at two concentrations (e.g., both 500 and 250 μ M). This allows more flexibility in dealing with drugs that may have high or low absorbance.

Guidelines for Setting Up the Assay, continued

- It is recommended that a given drug and concentration of that drug be tested in at least 4 wells to allow for potential outliers. Furthermore, the use of median values can often lead to more robust determinations than the average value since the median is not impacted by an extreme outlier.
- If using with UV/Vis spectroscopy to determine drug concentration, use UV-compatible microtiter plates (e.g., cat. no. MSCPNUV40).
- The typical detection wavelength for measuring drugs is 280 nm, but it is recommended that the detection wavelength be optimized so that it is in the linear range during calibration and actual measurement.
- When creating a calibration curve, a serial dilution is the easiest to construct. However, this is weighted for high concentrations. If the drug has low permeability, it may be advantageous to drop the points at the upper end of the calibration curve to get a more precise measurement at the lower end.
- Blank correct all samples using blank wells within the plate. Make sure that the blanks within the plate are highly consistent with one another.

Prepare samples

1. Prepare drug transport samples.
2. Prepare acceptor solution (buffer) of desired composition.
3. Prepare calibration standards. If using UV/Vis spectroscopy to assay samples, prepare the standards in exactly the same solutions as the drugs (i.e., include DMSO if your drugs are dissolved in DMSO).

Prepare Artificial Membrane

NOTE: Carry out steps 1 through 4 under a fume hood.

The following example creates a hexadecane artificial membrane. For additional information, see references 2, 3, 5, and 6. For information on lipid artificial membranes, see references 1, 4, and 7.

1. Prepare artificial membrane solution of 5% hexadecane in hexane (e.g., 200 μL hexadecane in 3800 μL hexane).
2. Remove the filter plate (donor plate) from the Multiscreen® Permeability Assembly and place it in the single well tray to protect the bottom of the filter wells during artificial membrane formation.
3. Apply 15 μL of 5% hexadecane/hexane solution to the center of each well in the 96-well donor plate to form the artificial membrane.
4. Allow plate to dry in hood for one hour.

Load Plates and Incubate

In order to balance the liquid levels, the recommended well volume is 125 μL for the donor plate and 200 μL for the acceptor plate. If using the PTFE acceptor plate (cat. no. MSSACCEPTOR) in place of the supplied acceptor plate, the recommended acceptor plate well volume is 300 μL .

NOTE: Handle plates carefully during assembly and disassembly to prevent air entrapment or cross-well contamination (spillover into adjacent wells).

1. Add 200 μL buffer to the acceptor plate wells.
2. With the donor plate still seated in the single well tray, add

Load Plates and Incubate, continued

125 μL of drug transport samples to donor plate wells.

3. Align the cut corners and carefully stack the donor plate on top of the acceptor plate, taking care not to entrap air or cause spillover into adjacent wells. Cover the donor plate with the supplied lid to minimize evaporation during extended incubation periods. When stacking assemblies, place the lid on the top donor plate only.
4. Incubate filter plate assembly at 25 °C for desired length of time.

NOTE: Incubation times can range from 5 to 16 hours depending on filter, artificial membrane composition, nature of compounds, and detection method sensitivity.

5. After incubation, remove the donor plate from the acceptor plate. Retrieve samples from both the donor and acceptor wells.

Sample Evaluation

1. Evaluate the solutions from both the donor and acceptor wells using a standard detection method such as a UV/Vis plate reader, HPLC/UV or LC/MS/MS.
2. Determine the permeability using these values. See formula below to calculate $\log(P_e)$.

$$\log P_e (\text{cm/s}) = \log \left[\frac{-\ln [1 - C_A / C_{\text{equilibrium}}]}{S(1/V_D + 1/V_A)t} \right]$$

where:

C_A = final drug concentration in the acceptor well (μM)

$C_{\text{equilibrium}}$ = theoretical equilibrium concentration

$$= [C_D \cdot V_D + C_A \cdot V_A] / [V_D + V_A]$$

where:

C_D = final drug concentration in the donor well (μM)

V_D = volume in the donor well (cm^3)

C_A = final drug concentration in the acceptor well (μM)

V_A = volume in the acceptor well (cm^3)

S = surface area (cm^2), typically 0.268 cm^2

V_D = volume in the donor well (cm^3)

V_A = volume in the acceptor well (cm^3)

t = incubation time(s)

NOTE: $C_{\text{equilibrium}}$ takes into account nonspecific binding and precipitation of drug and only includes the total soluble drug in the donor and acceptor compartments.

Specifications

Operating temperature	Ambient
Typical operating volume	Donor wells: 125 μL Acceptor wells: 200 μL
Filtration area	0.268 cm^2
Dimensions (length \times width \times height)	
Lid	127.6 \times 85.5 \times 9.0 mm (5.03 \times 3.37 \times 0.36 in.)
Donor plate	124.0 \times 81.9 \times 12.2 mm (4.88 \times 3.22 \times 0.48 in.)
Acceptor plate	126.2 \times 84.6 \times 10.5 mm (4.97 \times 3.33 \times 0.42 in.)

Specifications, continued

Single well tray	127.8 × 85.4 × 13.4 mm (5.03 × 3.36 × 0.53 in.)
Materials of Construction	Lid: Styrene Donor plate: Styrene with 0.4 µm polycarbonate membrane Acceptor plate: Polyethylene terephthalate glycol-modified Single well tray: Styrene

Device Storage

Store at room temperature for up to 3 years from date of manufacture. Refer to expiration date on package label.

References

1. Di, L., E. H. Kerns, K. Fan, O. J. McConnell, G. T. Carter. 2003. High throughput artificial membrane permeability assay for blood-brain barrier. *European Journal of Medicinal Chemistry* 38: 223–232.
2. Kellard, L. and Engelstein, M. 2007. Automation of cell-based and noncell-based permeability assays. *Journal of the Association for Laboratory Automation* 12(2):104–109. <http://journals.sagepub.com/doi/pdf/10.1016/j.jala.2006.10.008>
3. Wohnsland, F. and Faller, B. 2001. High-throughput permeability pH profile and high-throughput alkane/water log P with artificial membranes. *Journal of Medicinal Chemistry* 44(6):923–930. <http://pubs.acs.org/doi/pdf/10.1021/jm001020e>

The following references are available at www.millipore.com. Enter the publication number in the search box.

4. PC040EN00 – MultiScreen® Filter Plates for PAMPA
5. PC2004EN00 – HDM-PAMPA with the MultiScreen® Permeability Filter Plate
6. AN1725EN00 – MultiScreen® Permeability Plates: The evaluation of the reproducibility of passive, transcellular drug permeability assays
7. AN1728EN00 – Evaluation of the reproducibility of Parallel Artificial Membrane Permeation Assays (PAMPA)

Product Ordering Information

This section lists the catalogue numbers for the MultiScreen® Permeability Plate Assembly and related products. See the Technical Assistance section for contact information. You can purchase these products on-line at www.millipore.com/products.

Description	Catalogue No.	Qty/Pk
MultiScreen® Permeability 96-well Filter Plate Assembly (includes lid, donor plate, acceptor plate, and single well tray)	MPC4NTR10	10/pk
Related Products		
MultiScreen® _{HTS} 96-well UV-compatible Collection Plate	MSCPNUV40	40/pk
MultiScreen® Single-well Culture Tray, sterile	MAMCS0110	10/pk
MultiScreen® 96-well Deep Well Collection Plate	MDCPN2M50	50/pk
MultiScreen® Acceptor Plate	MSSACCEPTOR	1/pk

Notice

The information in this document is subject to change without notice and should not be construed as a commitment by Merck Millipore Ltd. ("Millipore") or an affiliate. Neither Merck Millipore Ltd. nor any of its affiliates assumes responsibility for any errors that may appear in this document.

Technical Assistance

For more information, contact the office nearest you. In the U.S., call 1-800-645-5476. Outside the U.S., 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.

Standard Warranty

The applicable warranty for the products listed in this publication may be found at www.millipore.com/terms ("Conditions of Sale").



The M logo, Millipore, and MultiScreen are registered trademarks of Merck KGaA, Darmstadt, Germany.

© 2017 EMD Millipore Corporation. Billerica, MA, U.S.A. All rights reserved.

PR04957, Rev. 01/17