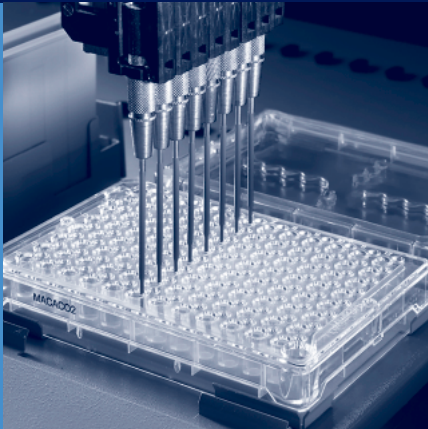


MultiScreen® Assay System for High Throughput Cell-based Transport



- ▶ Use manually or with automation
- ▶ Membrane optimized for cell growth and differentiation
- ▶ Single-well or 96-well feeding trays available for assay versatility
- ▶ Apical and basolateral access ports allow for easy access to both sides of cell monolayer

96-well assay system optimized for use with epithelial cells including Caco-2 and MDCK

Increase Screening Throughput

The patent-pending, 96-well design of the MultiScreen Caco-2 assay system is optimized for use with adherent cells including Caco-2 and MDCK. The membrane-based system promotes cell growth and good monolayer formation. The 96-well system also increases screening throughput 4 times over current 24-well systems and is fully automation compatible.

Automation protocols are available from Millipore at www.millipore.com/automation.

Comprehensive System Supports Growth to Analysis in One Plate

MultiScreen Caco-2 assay is designed as a complete system to support cell growth, feeding and analysis in one plate. The 96-well membrane-bottom plate fits together with a choice of a 96-well or single well feeding tray. At the time of trans-

port analysis, the membrane-bottom tray is simply transferred to a 96-well transport tray for analysis.

This streamlined design enhances compatibility with seed and feed systems, most liquid handlers (including Tecan and Cytogation, Inc.), and TEERs (transepithelial electrical resistance) 96-well measurement systems (WPI, Inc.).

Apical and Basolateral Access to Cells

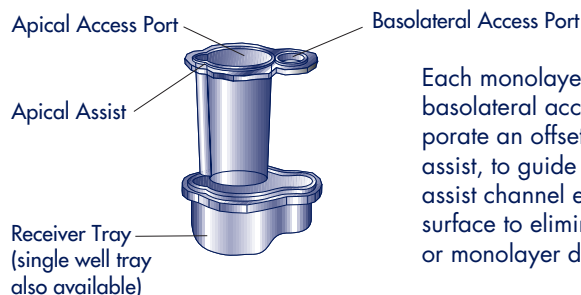
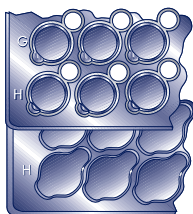
The patent-pending MultiScreen Caco-2 assay system is designed with apical and basolateral access ports for contamination-free access to cell monolayers, easy feeding and media changes, and effective sample analysis. The basolateral access ports are especially effective during transport rate analysis as there is no need to disassemble the assay system to sample basolaterally.

Each well and basolateral access hole is aligned to complement use with automated probes.

Plate Design Maintains Assay Integrity

The MultiScreen Caco-2 assay system design eliminates any need to detach tray assemblies for feeding, washing or media changes. This prevents monolayer disruption, contamination and membrane damage.

Top Down View (not assembled)



Each monolayer is accessible via apical and basolateral access ports. The wells also incorporate an offset apical channel, the apical assist, to guide manual pipette tips. The apical assist channel ends just short of the membrane surface to eliminate the chance of membrane or monolayer disruption while pipetting.

Performance

Figure 1. Drug compounds representing active, passive and efflux transporters were tested at ArQule, Inc. using the MultiScreen Caco-2 assay system (for a complete list of compounds, refer to Millipore Application Note AN1727EN00). Each compound's permeability rate was plotted against their percent human absorption values. A nonlinear regression curve was generated using Statistica™. The resultant curve provided a good fit to all of the data points to demonstrate that the MultiScreen Caco-2 assay system could correctly identify and classify the permeability properties of all 25 compounds tested.

Drug Permeability Rates Correlate to Human Absorption

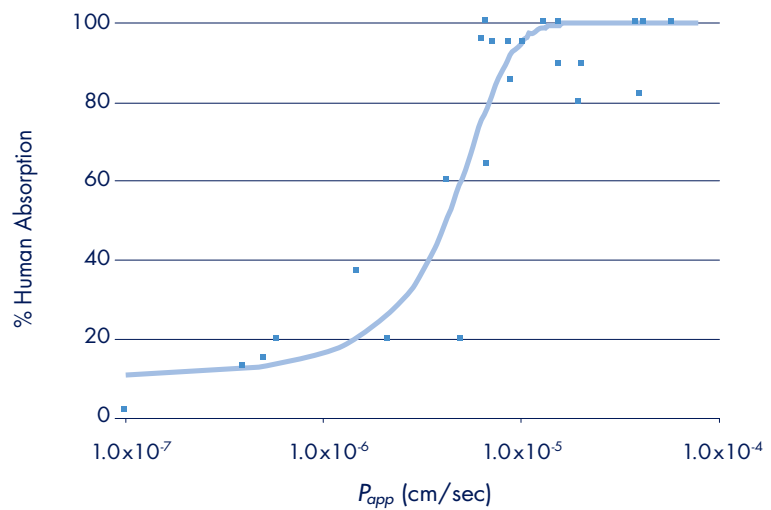


Figure 2. Caco-2 drug transport rates using 10 tritium labeled drugs were measured using the MultiScreen Caco-2 assay system and compared to the drug transport rates obtained from an established 24-well system. This set compares the results from four separate experiments. The R^2 value of 0.99 suggests that the data between the two formats (24-well assay vs. Millipore 96-well) correlate. For a complete listing of the drugs tested, refer to Millipore Application Note AN1727EN00.

Drug Permeability Rates Correlate with 24-well Assay Systems

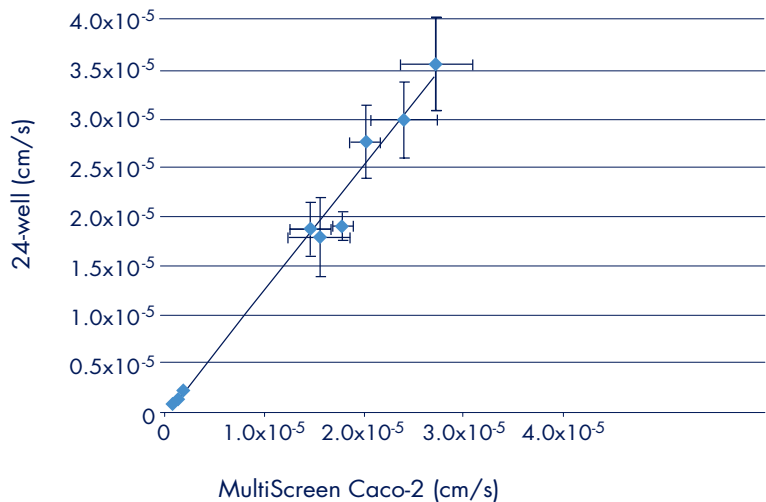


Figure 3. Low variability of in vitro cell-based assay platforms is critical for reliable assay results. This figure examines the reproducibility of drug transport rates in MultiScreen Caco-2 assay system using a 21-day culture of Caco-2 cells and a single drug, propranolol, in all 96-wells of every plate tested. Transport rates were determined in 3 experimental runs performed on different days, each run consisting of 4 different production lots of MultiScreen plates. Analysis of variance on the plate average, inter-plate and intra-plate standard deviations does not vary significantly among the production lot tests or days. The total standard deviation is calculated to be 0.3×10^5 cm/sec.

Reproducible Assay Results

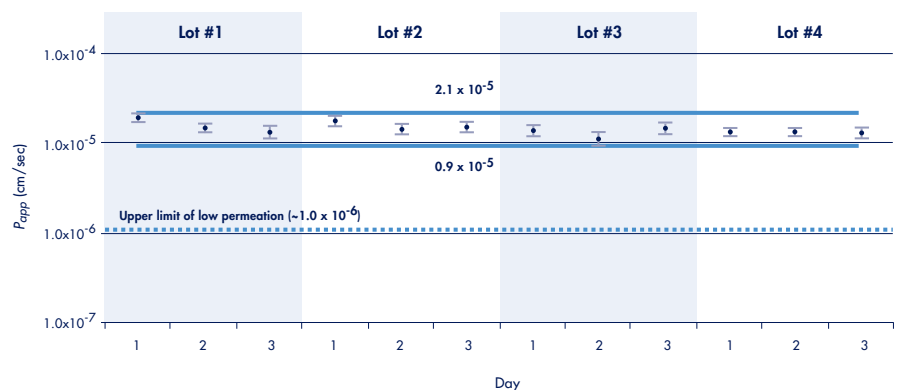


Table 1. The MultiScreen Caco-2 assay system incorporates an optimized membrane to promote cell growth for active digoxin transport assays. This table calculates the ratio of active transport rates (ratio of basolateral to apical/apical to basolateral) to illustrate the higher ratios demonstrated by MultiScreen Caco-2 10- and 21-day cultures. All results are the average of data from 8 wells.

Optimized Membrane Promotes Cell Growth to Yield Superior Active Transport Results

Digoxin transport ratios for 10- and 21-day cultures

BL → Ap/ Ap → BL Ratio	MultiScreen Caco-2 Assay System		Company B 96-well Plate	
	10 day	21 day	10 day	21 day
Digoxin	11.5	30.5	6.6	14.5
% LY rejection AP/BL	99	>99	98	>99

Table 2. Drug transport values (21-day) were compared for the MultiScreen Caco-2 assay system and a 96-well filter plate from company B using mannitol, digoxin, propranolol and testosterone. Results show that the optimized plate design and larger membrane surface area in the 96-well MultiScreen Caco-2 system yields a better dynamic range (MultiScreen P_{app} 0.5 – 26.3 vs. company B 96-well plate 0.4 – 17.5 x 10⁻⁶ cm/sec).

Optimized Plate Design Improves Dynamic Range in P_{app} Evaluation for Known Compounds

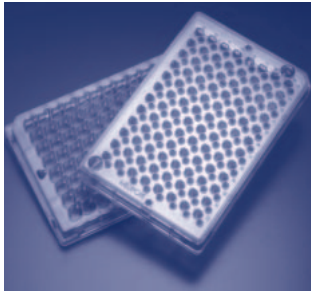
P_{app} (x10 ⁻⁶ cm/sec) Ap → BL	MultiScreen Caco-2		Company B 96-well Plate	
	P_{app}	s.d.	P_{app}	s.d.
Mannitol	1.1	0.2	1.2	0.2
Digoxin	0.5	0.1	0.4	0.2
Propranolol	6.6	0.6	2.7	0.5
Testosterone	26.3	3.6	17.5	4.1

Table 3. The lucifer yellow rejection achieved with 4 and 7 day MDCK cultures shows that monolayers were grown successfully on the MultiScreen Caco-2 assay system. MDCK cell differentiation is further evaluated by the high ratio of active transport rates.

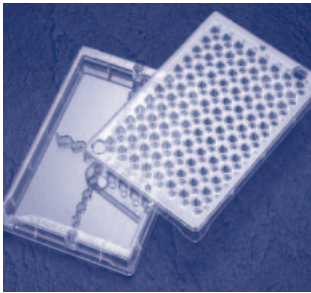
Assay System Supports MDCK Cell Monolayer Growth and Differentiation

LYR and digoxin transport ratios for 4 and 7 day cultures

	% Lucifer Yellow Rejection	Digoxin Transport Ratios (BL → Ap/ Ap → BL)
4 day	99.6 (n=5)	19.5 (n=5)
7 day	99.7 (n=9)	20.0 (n=7)



MultiScreen Caco-2 with 96-well feeder tray.



MultiScreen Caco-2 with single-well feeder tray.

Ordering Information

MultiScreen Caco-2 filter plates and assay systems are sold sterile. See ordering details for assembly details.

Description	System Components	Catalogue No.
MultiScreen Caco-2 assay system with 96-well feeder tray	– 2 growth assemblies (includes growth plate and 96-well feeder tray) – 2 transport trays with lids	MACA C02 S2
	– 5 growth assemblies (includes growth plate and 96-well feeder tray) – 5 transport trays with lids	MACA C02 S5
MultiScreen Caco-2 growth assembly with single well feeder tray	– 5 growth assemblies (includes growth plate and single-well feeder tray)	MACA C02 B5
Accessories		
96-well feeder/transport trays with lids	– 5 x 96-well trays with lids (can be used for feeding or transport analysis)	MACA COR S5

To Place an Order or Receive Technical Assistance

For additional information call your nearest Millipore office:

In the U.S. and Canada, call toll-free 1-800-MILLIPORE (1-800-645-5476)

In the U.S., Canada and Puerto Rico, fax orders to 1-800-MILLIFX (1-800-645-5439)

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