ReadyCell
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MedTech BARCELONA by Leitat

24-Well PreadyPort MDR1

User's Manual

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Product Description

PreadyPort is an *in vitro* cell-based model built on genetically-modified differentiated Mardin Darby Canine Kidney II (MDCKII) cells, forming a cell monolayer. Cells are seeded in 24 Transwell® inserts with semiporous (0.4 µm) polycarbonate (PC) membrane (CORNING Cat#3397) that separates an apical and a basal compartment.

PreadyPort MDR1 contains MDCKII cells transfected with the ABCB1 gene to overexpress the multidrug resistance protein 1 (MDR1), a membrane transporter of considerable clinical importance, to evaluate drug-transporter interactions in preclinical stages[1].

PreadyPort is delivered in a 24-well/plate format with a unique Shipping Medium (a gel-like cell culture medium) established by MEDTECH BARCELONA which enables the transport of cells at room temperature and in a ready-to-use format.

NOTE: *Other MDCKII cells overexpressing either other transporters or the empty vector (WT) may be included, if required.*

Intended Use

This product is mainly indicated for assessing:

- MDR1 substrates, inhibitors and inducers
- MDR1 transporter-based drug-drug interactions (concomitantly administered drugs)
- drug permeability by passive diffusion through a physiologically relevant barrier.

NOTE: *This cell-based model is intended for scientific research purposes only. Not for human or veterinary use.*

Principle

Passive permeability and outward active transport of drugs are carried out with MDCKII cells stably expressing pharmacologically relevant human drug transporters in the apical domain of the plasma membrane.
In the experimental setup, these cells will be differentiated on Transwell® inserts to form a tight cell monolayer that prevents media from wicking between the insert (apical compartment) and the plate well (basal compartment). Efflux transporters localized in the apical side will introduce a basolateral-to-apical bias in the distribution of substrate compounds between the two compartments.

In a standard assay design, the reaction is initiated by filling either compartment with the solution containing the test compound. The distribution is assessed over time by withdrawing and analyzing samples from both compartments. After normalization, the speed of translocation is obtained for both directions. The ratio of the two values is a measure of the passive and active transport mechanisms involved in the distribution of the compound.

Handling and experimental procedures are provided below. The manual has been written for users with experience in cell culturing and pharmacological drug discovery *in vitro* testing experiments. For more detailed advice and training opportunities, please contact us at:

reagents@medtechbcn.com

Timeline for Delivery and Experimental Procedures

- Day 1: Start of Production (Seeding of cells)
- Day 4: Pre-shipping Quality Control (TEER and Lucifer Yellow)
- Days 4-5: Package Dispatch (depending on destination)
- Days 6-7: Package Delivery
- Day 8: Replacement of Shipping Medium (liquefaction)
- Day 11: Quality Control Experiments, Medium Replacement
- Days 12-15: Assay Performance

Packages are dispatched on Mondays/Tuesdays and delivered within 24-48 h to EU countries, 48-72 h to USA, and 48-96 h to Asian countries. For other locations and customized schedules, please contact us at:

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The recommended timing overview for transport experiments is Day 12 (Tuesday) (see Figure 1 for details).

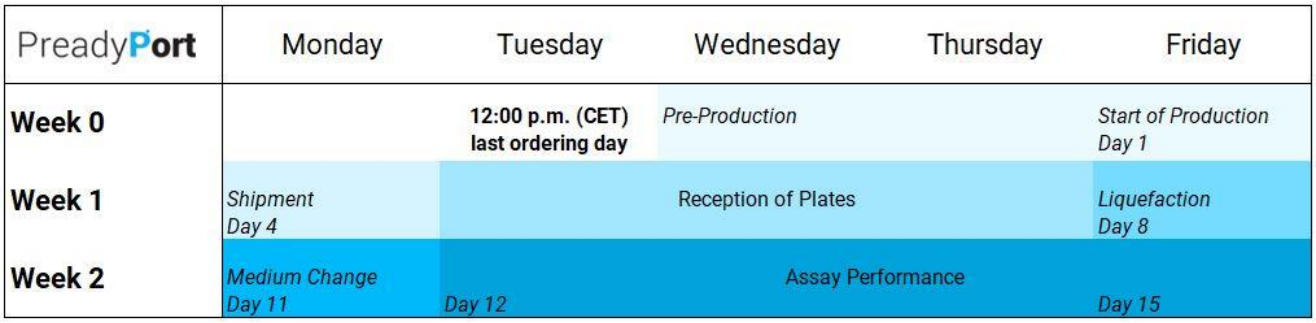


Figure 1. Timeline of manufacturing and operation for PreadyPort in 24-well format.

In case you choose to conduct the experiments later, TEER measurements and medium replacement must be carried out as follows:

DAY OF EXPERIMENT	TEER MEASUREMENT	MEDIUM REPLACEMENT
Tuesday (Day 12)	Monday (Day 11)	Monday (Day 11)
Wednesday (Day 13)	Monday (Day 11) Wednesday (Day 13)	Monday (Day 11)
Thursday (Day 14)	Monday (Day 11) Wednesday (Day 13)	Monday (Day 11) Wednesday (Day 13)
Friday (15)	Monday (Day 11) Wednesday (Day 13) Friday (15)	Monday (Day 11) Wednesday (Day 13)

Table 1. Recommended day for TEER measurement and medium replacement.

NOTE: *These steps enable the planning of the assay according to the user´s convenience.*
IMPORTANT NOTE: *TEER evaluation will be carried out on Monday (Day 11) before performing any further processing, including medium replacement. Based on our experience with long-distance shipments and/or extreme temperatures at destination, in case TEER values are low, it is recommended to perform a medium change and wait until Wednesday to let the cells recover. On Wednesday, read the TEER again and perform the assay accordingly.*

Equipment (not included)

- Cell culture laminar flow hood
- CO2 incubator
- Water bath
- Multichannel pipettes
- Automatic multichannel micropipette (recommended)
- Aspiration system
- **24-well format vacuum manifold (Drummond Cat# 3-000-097 recommended)**
- Trans-Epithelial Electrical Resistance (TEER) meter (WPI EVOM series)
- **24-well electrode** (**WPI STX100C recommended**)
- Fluorometer (Fluoroskan Ascent CF)
- Quantitative analytics equipment

Consumables

- **Reservoir plate (Corning Cat# 3524)** ***(not provided)***
- Sterile culture medium containers (i.e., Costar 50 ml, Cat# 4870) *(not provided)*
- 15 and 50 mL conical tubes and 1.5 mL Eppendorf tubes *(not provided)*
- Pipette tips *(not provided)*

Solutions (may be included)

NOTE: *MedTech Barcelona can supply Medium and Transport Buffer if required.*

- **MDCKII Cell Culture Medium**: Dulbecco's Modified Eagle's Medium (GIBCO Cat# 22320022) supplemented with (final concentrations):
  - 10 % V/V Fetal Bovine Serum (BIOWEST Cat# DE14-801F)
  - 100 U/mL; 0.1 mg/mL Penicillin-Streptomycin (LONZA Cat# DE17-602F)
  - 1x MEM non-essential amino acid solution (GIBCO Cat# 11140035)
- **Transport Buffer solution**: Hank's 1X Balanced Salt Solutions (HBSS 1x) (HyClone Cat# SH30268)
- **Recommended reporter Substrate**: Digoxin (SIGMA Cat# 04599)
- **Recommended reporter Inhibitor**: Valspodar (PSC833) (SIGMA Cat# SML0572)

NOTE: *If the specified reagents are not available, other reagents with similar features and specifications can be used.*

Handling

Upon reception, retrieve the zipped bags containing the plates. Open the zip and leave the bag in a dark location at room temperature until Day 8 (refer to Timeline; Figure 1).

Replacement of Shipping Medium

CAUTION: *Never handle more than one plate at a time while changing the shipping medium. Re-solidification of the shipping medium may damage the cell monolayer.*

These **steps** will be **carried out on Day 8** (refer to Timeline; Figure 1). Perform all manipulation under sterile conditions.

1. Retrieve the plates from the bags and remove the parafilm wrap.
2. **Incubate** the plates in a 5 % CO2 humidified atmosphere at 37 °C for **4 hours**, until the **shipping medium** reaches **liquefaction**.
3. Remove one PreadyPort plate from the incubator and place it inside the laminar flow hood, along with one reservoir plate.
4. Using sterile procedures (**inside the laminar flow hood**), fill a sterile reagent reservoir with 50 mL of pre-warmed (37 °C) MDCKII cell culture medium.
5. Open the PreadyPort plate and the reservoir plate, and leave their lids upwards, next to the plates.
6. Carefully lift the 24-integrated apical compartments of the PreadyPort plates and transfer them onto the reservoir plate.
7. Remove all liquefied shipping medium from the basal compartments of the PreadyPort plate via aspiration with the 24-well manifold.
8. Using a multichannel pipette, dispense **900 µL** of MDCKII cell culture medium from the sterile reservoir, and fill, the **basal compartments** of the PreadyPort plate, column by column.
9. Using the aspiration manifold connected to a vacuum pump (adjust aspiration flux to medium-low), aspirate the liquefied shipping medium from the apical integrated inserts of the PreadyPort plate, taking care not to disrupt the monolayer. Make sure the shipping medium has been removed from all wells. Approximately 50 µL of medium will be left in each well.
10. Using a multichannel pipette, dispense **300 µL** of MDCKII cell culture medium from the sterile reservoir, and fill, the **apical compartments** of the PreadyPort plate, column by column. Always add the medium against the wall of the well, and not directly onto the cell monolayers.
11. Carefully return the apical inserts onto the basal compartment of the PreadyPort plate. Replace the lid and place it inside the cell culture incubator, set at 37 °C and 5 % CO2.
12. Once the shipping medium has been substituted by fresh MDCKII cell culture medium, the plates should be placed inside the incubator until next Monday (Day 11). **Replacement with a new fresh medium** will be carried out once (Day 11) or twice (Days 11 and 13) depending on the day of the assay (refer to Table 1 for details), following the procedure described above.

NOTE: *Do not discard the reservoir plate, as it will be used in the permeability assay.*

Quality Control of the Barrier System

Pre-assay Quality control – TEER Measurement

This section provides general instructions for TEER evaluation. It is important to read carefully the instructions of the TEER measurement equipment in conjunction with these instructions.

The timeline for TEER evaluation is detailed in Table 1. TEER measurement will be carried out before performing any further processing, including the experiment and the medium replacement.

NOTE: *Never perform the TEER measurement with the shipping medium. Do not repeat TEER measurements in the same well.*

For **TEER evaluation**, follow the steps below:

1. **Sterilize the electrode** (probe) by submerging both tips in 70 % ethanol for 5 minutes.
2. Equilibrate the electrode (probe) for 5 minutes in MDCKII cell culture medium, **pre-warmed at room temperature**.
3. While the electrode is equilibrating, remove the PreadyPort plate from the incubator and place it in a laminar flow hood. **Allow the plate to reach room temperature** (approximately 20 minutes), as TEER measurements should be performed under this condition.
4. If using chopstick **electrodes** like the STX2 (WPI EVOM series), place the probe into the insert system, so that the thinner electrode is within the narrowest slit, which corresponds to the basal part of the inserts. The thicker electrode must be placed inside the widest slit, corresponding to the apical part of the inserts. Both electrodes have to be **well submerged** within the cell culture medium of the apical and basal compartments **for a correct evaluation**. Be careful **not to touch the cell monolayers**!

   NOTE: *It is highly recommended to use the WPI STX 100C electrode to prevent cell damage. Watch out to set the electrode in the right position.*

5. Record the resistance readout in ohms (Ω) for each well. **TEER value is the result of multiplying the resistance value by the cell growth area (cm^2)**.

Acceptance Criterion

Active membrane surface (Corning and Falcon plates)	**0.33 cm2**
TEER value	**> 75 Ω x cm2**

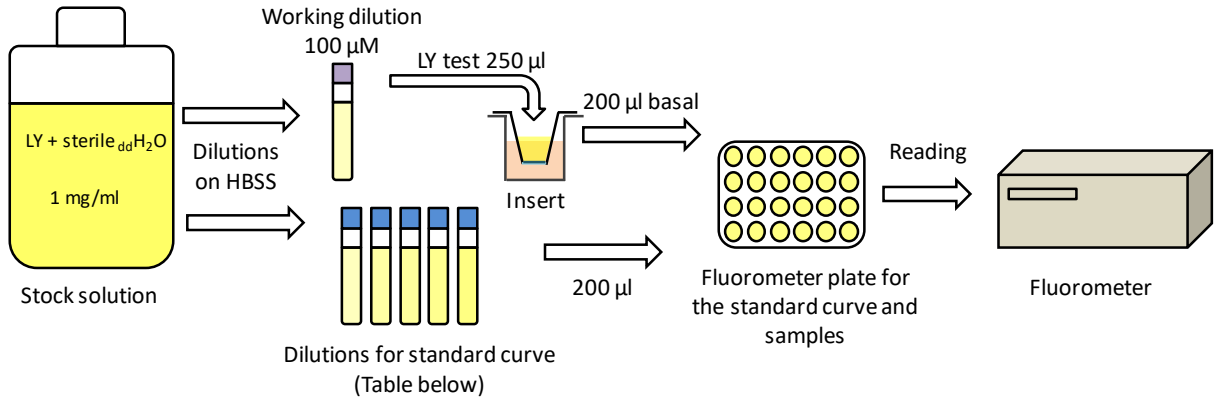
Post-assay Quality Control – Lucifer Yellow (LY) Paracellular Permeability Assay

Prepare a **1 mg/mL (2.187 mM) LY solution** in sterile ddH_20. Make aliquots (e.g., 500 µL) and store them at -20 °C.

Dilute LY stock solution in transport assay buffer to a **100 µM final concentration**. Working dilution will be used to prepare the calibration curve and for the LY test (see Figure 2 for details).

To proceed with the LY permeability assay, follow the steps below:

1. Prewarm the 100 µM working LY solution at 37 °C covered with foil to protect it from light.
2. **Prepare the calibration curve** by making serial 1:2 dilutions of the working solution (see Figure 2).
3. **Rinse both** the apical and the basal **compartments** gently with transport assay buffer following the procedure described in "Replacement of Shipping Medium" (steps 4-11). Instead, use a volume of **250 µL and 750 µL** for the apical and basal compartments, respectively.
4. Remove the transport assay buffer from the apical and basal compartments following the same procedure.
5. Add **250 µL of 100 µM LY** working dilution into the **apical compartment**.
6. Add **750 µL of transport assay buffer** to the **basal compartment**.
7. **Incubate** the PreadyPort plate, protected from light, in the cell incubator (at 37 °C and 5 % CO_2) for **1 h**.
8. **Take 200 µL** from the **basal compartment and** from the **calibration curve,** and load them into an empty 96-well plate for fluorescence-based assays. Mix well and avoid bubble formation when getting samples and standards!
9. **Read the fluorescence** intensity in a fluorometer at **485/527** excitation/emission wavelengths.



0 µM BLANK	*0.048 µM*	*0.097 µM*	*0.195 µM*	*0.390 µM*	*0.781 µM*	*1.562 µM*	*3.125 µM*	*6.25 µM*	*12.5 µM*	*25 µM*	*50 µM*	*100 µM*

Figure 2. General procedure for LY permeability assay and recommended concentrations for the calibration curve.

Acceptance Criterion

LY Paracellular Flux	**≤ 2 %**
LY apparent permeability (Papp)	**≤ 4.2 x 10-6 cm/s**

General Protocol for Transport Assays

General Considerations

PreadyPort is designed for conducting permeability *in vitro* assays of established and investigational compounds in order to predict their interaction with membrane-associated proteins (transporters). Specifically, this cell-based model is optimized for the identification of substrates and/or inhibitors and inducers of MDR1.

Cell monolayers may also be used to assay drug permeation across a pharmacological barrier.

Recommended Reference Compounds

The compounds listed below (also referenced in the "Solutions" section) are recommended for the assay as a reference substrate and inhibitor of the MDR1 transport protein.

- Reporter MDR1 Substrate: Digoxin (SIGMA Cat# 04599)
- Reporter MDR1 Inhibitor: Valspodar (PSC833) (SIGMA Cat# SML0572)

Sample Plate Layout

The PreadyPort 24-well format allows evaluating the permeability of 1 compound in triplicate in the A-B/B-A directions following the recommended plate layout shown below.

	A-B			B-A		
	1	2	3	4	5	6
A	Digoxin_R1	Digoxin_R2	Digoxin_R3	Digoxin_R1	Digoxin_R2	Digoxin_R3
B	Dig/Valsp_R1	Dig/Valsp_R2	Dig/Valsp_R3	Dig/Valsp_R1	Dig/Valsp_R2	Dig/Valsp_R3
C	Comp 1_R1	Comp 1_R2	Comp 1_R3	Comp 1_R1	Comp 1_R2	Comp 1_R3
D	Comp 1/Inh_R1	Comp 1/Inh_R2	Comp 1/Inh_R3	Comp 1/Inh_R1	Comp 1/Inh_R2	Comp 1/Inh_R3

R = replicate

Figure 3. Recommended sample plate layout to investigate MDR1-mediated transport and potential drug-transporter interactions.

- Initial concentration suggested for unknowns: 10 µM
- Replicates: 3
- Time points: 0 and 2 h
- Volumes: *Apical compartment*: 250 µL
  Basal compartment: 750 µL

NOTE: *The procedure should be undertaken in biosafety level II containment standards to ensure sterile conditions. Assay transport buffer solution should be pre-warmed to 37 °C to avoid temperature stress. Do not use LY and tested compounds concomitantly in the same well. LY may interfere with certain substances, resulting in false data.*

Protocol

The following protocol applies for one plate seeded with MDR1-expressing MDCKII cells and those transfected with the empty vector (WT).

*The **general procedure for permeability assays** in the A-B/B-A directions is represented in **Annex I**.*

Apical-to-Basal Studies

Test compounds are applied to the apical side of the cell monolayer (upper compartment of the insert), and the **apical-to-basal (A-B)** transport through the cell barrier is evaluated by sample recovery and test compound detection in the basal (lower) compartment over a defined incubation period. A-B permeability of test compounds is determined as the coefficient of apparent permeability (P_{app}) in cm/s.

Preparation

1. **Prepare stock solutions of reference and tested compounds** in transport buffer. In case of poorly water-soluble compounds, DMSO may be used as a solvent. If so, it is recommended to keep the percentage of DMSO in the assay buffer below 1 %.
2. **Prepare working solutions of unknowns and reference compounds** in transport buffer. Substrates and inhibitors are mixed simultaneously in the working solution when both compounds are concomitantly assayed.
3. Fill a reagent reservoir with pre-warmed (37 °C) transport buffer.
4. Take the reservoir plate into the laminar flow hood.
5. **Remove** one **PreadyPort plate from the cell incubator** and place it beside the reservoir plate. Both plates should be oriented the same way.

Washing Steps

6. Open the PreadyPort plate and the reservoir plate, and leave the lids upwards next to the plates.
7. Carefully lift the 24 **apical inserts** of the **PreadyPort plate** and **transfer** them to the **reservoir plate**.
8. Using the 24-well manifold, **aspirate the cell culture medium** from the lower compartments of the PreadyPort plate.
9. Using a multichannel pipette, **fill**, column by column, each of the 24 wells of the **lower compartments** of the PreadyPort plate with **750 µL** of pre-warmed (37 °C) **transport buffer**.
10. Using the 24-well manifold, aspirate the cell culture medium of the apical inserts of the PreadyPort plate. Place the manifold perpendicular to the cell monolayer and close to the insert wall to avoid disturbing the cell monolayer.
11. Using a multichannel pipette, **fill**, column by column, each of the 24 apical inserts of the **upper compartment** of the PreadyPort plate with **250 µL** of pre-warmed (37 °C) **transport buffer**.
12. Carefully **return** the 24 **apical inserts** onto the wells of the basal compartment of the PreadyPort plate (**original position**).
13. Incubate the plate for **1 minute at room temperature** inside the laminar flow hood.
14. **Repeat steps 6 to 12 twice**. The first time, incubate the plate as performed in step 13. The **second time**, **incubate** the plate for **30 minutes** in the **cell incubator** (37 °C, 5 % CO_2).

NOTE: *Use low-medium suction power to avoid disrupting the cell monolayer.*

Transport Assay

15. **Take the plate** from the incubator, return it to the laminar flow hood and place it next to the reservoir plate. Both plates should be oriented in the same way.

16. Carefully lift the 24 **apical inserts** of the **PreadyPort plate** and **transfer** them to the **reservoir plate**.
17. Using the 24-well manifold, **aspirate the transport buffer** from the **lower compartment** of the PreadyPort plate.
18. Using a multichannel pipette, **fill**, column by column, each of the 24 wells of the lower compartments of the PreadyPort plate with **750 µL** of pre-warmed (37 °C) **transport buffer**.
19. Using the 24-well manifold, **aspirate** the **transport buffer** of the **apical inserts** of the PreadyPort plate. Place the manifold perpendicular to the cell monolayer and close to the insert wall to avoid disturbing the cell monolayer.
20. Add **275 µL of working solutions** (see sample layout in Figure 3 for details) to the 24 **apical inserts** of the PreadyPort plate. Immediately after (0 hours), **recover 25 µL (t0)** and keep them at -20 ºC until further analysis is performed.
21. Carefully **return** the 24 **apical inserts** onto the wells of the basal compartment of the PreadyPort plate (**original position**) and **leave** the plate in the **cell incubator** (37 °C, 5 % CO2) for **2 hours**. Shorter or longer periods of incubation may be required for very high or low permeability compounds.

NOTE: *Use low-medium suction power to avoid disrupting the cell monolayer.*

Sample Collection

22. **Take the plate** from the incubator, return it to the laminar flow hood and place it next to the reservoir plate. **Transfer** the **apical inserts** onto the **reservoir plate**.
23. Recover **25 µL** from the **apical inserts (t2h apical)** and the **lower compartments (t2h basal),** and keep them at -20 °C until further analysis is performed.
24. **Analyze all samples** using mass spectrometry according to your analytical procedures for tested and reference compounds.
25. **Calculate the compound apparent permeability (P_{app})** coefficient as indicated in the following section ("Evaluation of Compound Permeability").

Basal-to-Apical Studies

Test compounds are applied to the basal side of the cell monolayer (lower compartment of the insert), and the **basal-to-apical (B-A)** transport through the cell barrier is evaluated by sample recovery and test compound detection in the apical (upper) compartment over a defined incubation period. B-A permeability of test compounds is determined as the coefficient of apparent permeability (P_{app}) in cm/s.

All **steps are identical** to those described for the apical-to-basal studies, **except for the volumes added in steps 18 and 20**:

Step 18. Using a multichannel pipette, fill, column by column, each of the 24 wells of the **upper compartment** of the PreadyPort plate with **250 µL** of pre-warmed (37 °C) **transport buffer**.

Step 20. Add **775 µL of working solutions** (see sample layout in Figure 3 for details) to the **basal compartments** of the PreadyPort plate. Immediately after (0 hours), **recover 25 µL (t0)** and keep them at -20 °C until further analysis is performed.

NOTE: *At the end of the transport assay (A-B/B-A directions) perform the post-assay quality control as indicated in the section "Quality control of the barrier system".*

Evaluation of Compound Permeability

Apparent Permeability Coefficient (P_{app})

The transport efficiency of test substances and reference compounds is evaluated in each sample through P_{app} calculation, which is defined as follows:

$$P_{app} = \frac{dQ}{dt} \times \frac{1}{A \times C_0}$$

where P_{app} represents the coefficient of apparent permeability (in cm/s), which corresponds to the proportion of test compound that crosses the barrier at each time point (dQ/dt in nmol/s), divided by the product of the crossed area (A in cm^2) by the initial concentration of test compound (C_0 in nmol/ml) applied to the apical (A-B) or basal (B-A) compartments.

Considerations for calculations:

- When plotting Q versus time, consider the amount of material lost in previous stages
- A single time point can only be used in the linear range. Otherwise, the P_{app} value will be an underestimation of the real value. Sampling compounds with unknown behavior at a single time point is not recommended.

Efflux Ratio (ER)

The efflux ratio is a general measure of the involvement of active processes in compound permeability. This value results from dividing the compound P_{app} in the B-A direction by the P_{app} in the A-B direction.

$$ER = \frac{P_{app\ B\text{-}A}}{P_{app\ A\text{-}B}}$$

Mass Balance

Material balance calculation for each compound is determined as follows:

$$\text{Mass Balance (\%)} = \left[\frac{((Cap@time(i) \times Vap) + (Cbs@time(i) \times Vbs))}{Cinit@time0 \times V} \right] \times 100$$

Where

- Cap@time(i) and Cbs@time(i) correspond to test compound concentrations in pmols/mL at time (i) in the apical and basal compartments, respectively.
- Vap and Vbs are apical and basal volumes in mL.
- Cinit@time0 is the initial concentration of the test compound in pmols/mL at t0.
- V is the volume of either the apical compartment (A-B direction) or the basal compartment (B-A direction).

Data for Reference Compounds

Normal values and ranges for reference substances (according to FDA guidelines[1] and MEDTECH BARCELONA's internal data) are detailed below:

- TEER values > 75 Ω x cm^2 and LY paracellular flux values ≤ 2 % are strong indicators of cell barrier integrity.
- The efflux ratio of MDR1 substrate must be greater than 2 in those cells overexpressing the transporter.
- In the presence of an MDR1 inhibitor, the substrate's efflux ratio must decrease significantly (> 50 %).
- Material balance (mass balance) range must be between 80 % - 120 %.

PreadyPort MDR1						PreadyPort WT					
Substrate	Inhibitor	Concentration (µM)	Papp (x 10^{-6} cm/s)		ER	Substrate	Inhibitor	Concentration (µM)	Papp (x 10^{-6} cm/s)		ER
			A-B	B-A					A-B	B-A	
Digoxin	---	10	1.10 ± 0.21	12.11 ± 3.86	10.84 ± 2.16	Digoxin	---	10	2.22 ± 0.95	3.70 ± 1.34	1.90 ± 0.93
Digoxin	Verapamil	10:100	3.19 ± 0.39	4.89 ± 0.74	1.53 ± 0.10	Digoxin	Verapamil	10:100	2.83 ± 0.70	2.65 ± 0.34	0.99 ± 0.35
Quinidine	---	1	1.64 ± 0.25	49.93 ± 10.22	30.31 ± 1.68	Quinidine	---	1	12.58 ± 4.01	13.57 ± 5.39	1.06 ± 0.08
Quinidine	Valspodar	1:10	14.01 ± 4.43	20.84 ± 0.97	1.57 ± 0.40	Quinidine	Valspodar	1:10	9.21 ± 4.12	18.81 ± 3.25	2.25 ± 0.81

Table 2. Reference values for the MDR1 substrates (digoxin and quinidine) and inhibitors (verapamil and valspodar) incubated either in MDCKII cells overexpressing the MDR1 transporter (PreadyPort MDR1) or in those expressing the empty vector (PreadyPort WT).
Results for digoxin and quinidine are the mean of 3 independent experiments, respectively.

References

[1] Food and Drug Administration (FDA) (2020). *In Vitro Drug Interaction Studies — Cytochrome P450 Enzyme- and Transporter-Mediated Drug Interactions Guidance for Industry.* U.S. Department of Health and Human Services, Center for Drug Evaluation and Research (CDER). https://www.fda.gov/regulatory-information/search-fda-guidance-documents/vitro-drug-interaction-studies-cytochrome-p450-enzyme-and-transporter-mediated-drug-interactions

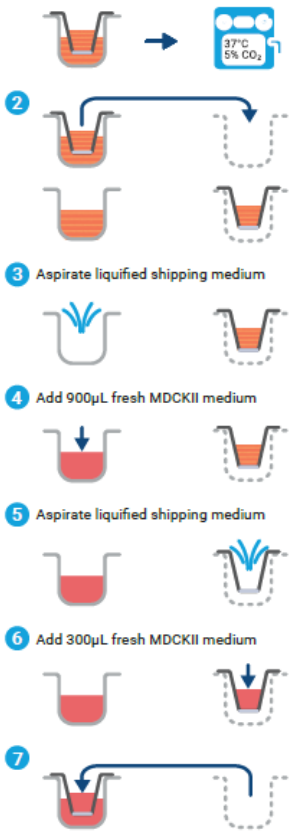
Annex I

ReadyCell PreadyPort 24 MDR1 Well Plate Instructions

A) Liquefaction and Shipping Medium Exchange (4h)

Reagents / Equipment

1. Liquefaction for 4h
2.
3. Aspirate liquified shipping medium
4. Add 900µL fresh MDCKII medium
5. Aspirate liquified shipping medium
6. Add 300µL fresh MDCKII medium
7.

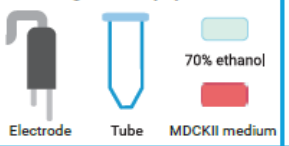


Important Notes

Key Dates	
Shipping Medium Exchange	Friday
TEER Measurement	Monday
MDCKII Medium Change	Monday
Assay	Tuesday

B) Pre-Assay Control TEER Measurement (1.5-2h)

Reagents / Equipment



1a. 70% ethanol 5 min to sterilize; ~5 min to equilibrate electrodes in pre-warmed medium

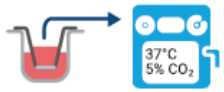
1b. ~20 min to equilibrate to room temperature

2. Measure TEER

3. Replace medium with fresh MDCKII medium

Add 900µL MDCKII Add 300µL MDCKII

4. Transfer to incubator and incubate for 24h

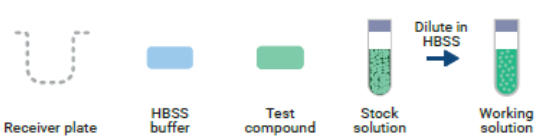


Important Notes

Key Data	
Well Area	0.33 cm²
Minimum TEER Values	75 ohms·cm²

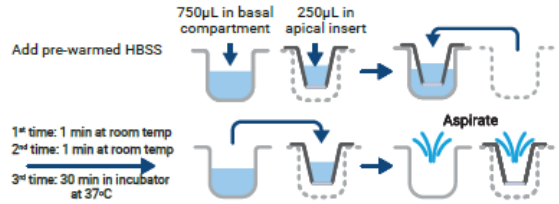
Permeability Assay (4h)

Reagents / Equipment



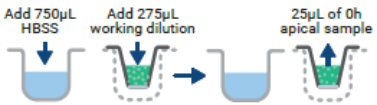
1. 

2. **Repeat 3x**



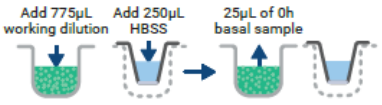
Select one assay to move forward

3. 1) Apical to Basolateral Assay



4. 

3. 2) Basolateral to Apical Assay



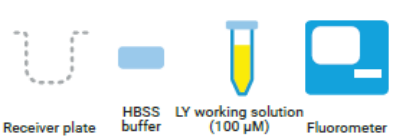
4. 

Important Notes

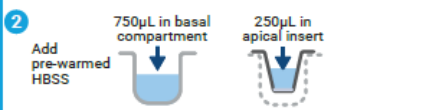
Assay Conditions	
Recommended Concentration (Unknowns)	10 µM
Replicates	3
Time Points	2 (0 and 2h)

D) Post-Assay Quality Control: Lucifer Yellow Paracellular Permeability Assay (2h)

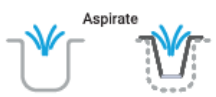
Reagents / Equipment

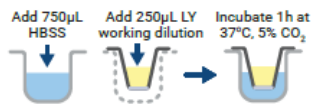


1. 

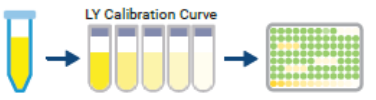
2. 

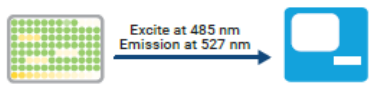
3. 

4. 

5. 

6. 

7. 

8. 

Important Notes

Lucifer Yellow Quality Control Parameters	
Paracellular Flux	≤2%
Papp	4.2x10⁻⁶ cm/s

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