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24-Well PreadyPort BCRP

User's Manual

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Table of contents

Product Description

PreadyPort is an in vitro cell-based model built on genetically-modified differentiated Madin-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 BRCP contains MDCKII cells transfected with the ABCG2 gene to overexpress the breast cancer resistance protein (BCRP), a membrane transporter of considerable clinical importance, to evaluate drug-transporter interactions in preclinical stages1.

PreadyPort is delivered in a 24-well 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.

Intended Use

This product is mainly indicated for assessing:

- BCRP substrates, inhibitors and inducers
- BCRP 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 or Sodium Butyrate Induction
- Day 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 reagents@medtechbcn.com.

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

PreadyPort	Monday	Tuesday	Wednesday	Thursday	Friday
Week 0		**12:00 p.m. (CET) last ordering day**	*Pre-Production*		*Start of Production Day 1*
Week 1	*Shipment Day 4*	Reception of Plates			*Liquefaction Day 8*
Week 2	*Medium Change Day 11*	*Day 12*	Perform Assay		*Day 15*

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

According to the day you choose to conduct the experiments, TEER measurement, medium replacement, and sodium butyrate induction must be carried out as follows:

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

Table 1. Recommended day for TEER measurement, medium replacement, and sodium butyrate induction.

***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 and sodium butyrate induction. 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. If so, perform the assay either on Thursday or Friday (see Table 1 for details).*

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 EVM-EL-03-03-04 recommended)**
- Fluorometer (Fluoroskan Ascent CF)
- Quantitative analytics equipment

***NOTE**: An electrode adaptor may be required depending on the model of the TEER meter you are using. Please refer to WPI for concerns regarding compatibility.*

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)
- **Post-assay quality control**: Lucifer Yellow (LY) (SIGMA L0259)
- **Induction of BCRP expression**: 1 mM Sodium Butyrate Solution (MERCK Cat# TR-1008-G)
- **Recommended reporter Substrate**: Prazosin (SIGMA Cat# P7791)
- **Recommended reporter Inhibitor**: Ko143 (SIGMA Cat# K2144)

***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, plates should be placed inside the incubator until next Monday (Day 11). **Replacement with a new fresh medium and/or sodium butyrate induction** will be carried out 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 carefully read 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, medium replacement, or sodium butyrate induction.

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 plates)	*0.33 cm2*
TEER value	*> 75 Ω x cm2*

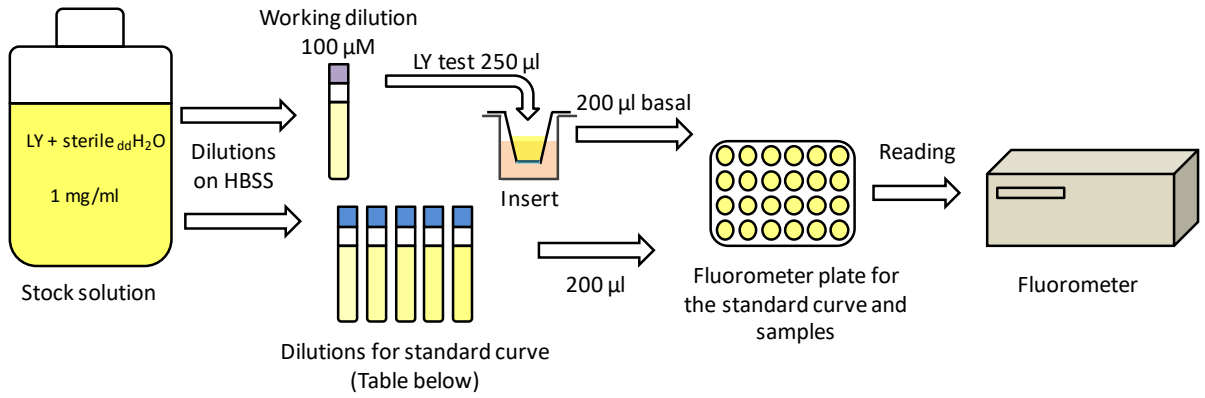
Post-assay Quality Control – Lucifer Yellow (LY) Paracellular Permeability Assay

Prepare a **1 mg/mL (2.187 mM) LY solution** in sterile ddH_2O. Make aliquots 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 BCRP.

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 BCRP transport protein.

- **Reporter BCRP Substrate**: Prazosin (SIGMA Cat# P7791)
- **Reporter BCRP Inhibitor**: Ko143 (SIGMA Cat# K2144)

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	Prazosin_R1	Prazosin_R2	Prazosin_R3	Prazosin_R1	Prazosin_R2	Prazosin_R3
B	Praz/Ko143_R1	Praz/Ko143_R2	Praz/Ko143_R3	Praz/Ko143_R1	Praz/Ko143_R2	Praz/Ko143_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 BCRP-mediated transport and potential drug-transporter interactions.
Comp (compound), Inh (inhibitor).

- 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.*