

Extract of the USER'S MANUAL

PREADYPORT

MDR1

24-transwell inserts or 96-multiwell inserts with differentiated MDR1-expressing MDCKII cells with differentiated MDR1-expressing MDCKII cells

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Timelines for Delivery and Experimental Procedures

- Day 1: Package Dispatch
- Days 2-3: Package Delivery
- Day 4: Replacement of Shipping Media
- Day 7: Quality Control Experiments, Media Replacement
- Days 8-11: Transport Experiments

Packages are dispatched **Tuesdays** and are delivered within two days in EU countries.

The recommended timing for transport experiments is **Day 8**. If you choose to conduct the experiments later, replacement of media on the filters the day before is recommended.

Equipment Not Included

- Cell culture biosafety cabinet
- CO₂ incubator
- Aspiration system
- Automatic multichannel pipettes
- for 24-well plates: 24-well format vacuum manifold (Drummond, cat. No 3-000-097 recommended)
- for 96-well plates: 96-well format vacuum manifold (Drummond, cat. No 3-000-093 recommended)
- Trans-Epithelial Electrical Resistance (TEER) meter (WPI EVOM with STX2 electrode recommended)
- Quantitative analytics equipment

Consumables Not Included

- for 24-well plates: 24-well Receiver plates (Costar cat. No 3526)
- for 24-well plates: 96-well Transport Analysis plate (Receiver plate) (Millipore cat. No PSHT004S5)
- Containers for cell culture medium and transport reagents (Costar cat. No. 4870 recommended)
- Pipette tips

Material Not Included

- **Cell Culture Media.** Supplement Dulbecco's Modified Eagle's Medium with these materials (final concentration):
 - Glucose (20 mM)
 - HEPES (25 mM)

- Fetal Calf Serum (10% V/V)
- L-glutamine (2 mM)
- PenStrep (100 U/mL; 0.1 mg/mL)
- MEM non-essential amino acid solution (1X)
- **Conditioning Buffer.** HBSS with $\text{Ca}^{2+}/\text{Mg}^{2+}$. We recommend Sigma ready-to-use buffer (cat. No H8264) or equivalent. In case you prefer in house preparation, dissolve these chemicals in 900 mL purified water (dry powder weight):
 - KCl (0.4 g)
 - KH_2PO_4 anhydrous (0.06 g)
 - NaHCO_3 (0.35 g)
 - NaCl (8 g)
 - Na_2HPO_4 anhydrous (0.048 g)
 - D-Glucose (1.0 g)

Dissolve $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (0.185 g) and MgSO_4 anhydrous (0.098 g) separately in 20 mL each and add to buffer drop by drop while stirring.

Bring total volume to 1 L and sterilize by filtering. Store at 4°C until use. Do not use beyond five days after sterilization.

- **Transport Buffer.** HBSS with $\text{Ca}^{2+}/\text{Mg}^{2+}$, with HEPES. Supplement 1 L of HBSS with $\text{Ca}^{2+}/\text{Mg}^{2+}$ buffer (see above) with 2.98 g of HEPES. Set pH to 7.4 and sterilize. Store at 4°C until use.
- **Reporter substrate.** We recommend digoxin for the PreadyPort™-MDR1 product.
- **Inhibitor:** We recommend PSC833 for the PreadyPort™-MDR1 product.