MedTech BARCELONA by Leitat

ReadyCell
EASING YOUR SCREENING

96-We PreadyTake OATP1B3

User's Manual

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Table of contents

Product Description

PreadyTake is an in vitro cell-based model built on genetically-modified differentiated Human Embryonic Kidney 293 (HEK293) cells, forming a cell monolayer.

PreadyTake OATP1B3 contains HEK293 cells transfected with the SLCO1B3 gene to overexpress the organic anion-transporting polypeptide 1B3 (OATP1B3), a membrane transporter of considerable clinical importance, to evaluate drug-transporter interactions in preclinical stages[1].

PreadyTake OATP1B3 has been designed in a 96-well plate format (see Figure 2). Half of the plate (n=30 wells) is seeded with OATP1B3-expressing HEK293 cells (HEK-OATP1B3) while the other half plate (n=30 wells) is seeded with the cells transfected with the empty vector (named either as HEK-MOCK cells or HEK-WT cells).

PreadyTake is delivered in a 96-well plate with a unique Shipping Medium (a gel-like cell culture medium) established by MEDTECH BARCELONA which enables cell transportation at room temperature and in a ready-to-use format.

***NOTE**: Other HEK293 cells overexpressing other transporters may be included, if required. Similarly, additional seeding plate configurations can be considered.*

Intended Use

This product is mainly indicated for *in vitro* evaluation of:

- OATP1B3 substrates, inhibitors and inducers
- OATP1B3 transported-based drug-drug interactions (concomitantly administered drugs)
- Competitive inhibition (unexpected drug elimination)

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

Principle

Uptake transporter in vitro assays are carried out with cell lines stably expressing pharmacologically relevant human transmembrane receptors. Drug-transporter interaction involving the drug candidate as a substrate or an inhibitor of the transporter protein is evaluated by comparing compound accumulation in cells overexpressing the transmembrane protein and non-specific accumulation in those expressing the empty vector.

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, please contact us at:

reagents@medtechbcn.com.

Timeline for Delivery and Experimental Procedures

- Day 1: Start of Production (Seeding of cells)
- Days 4-5: Package Dispatch (depending on destination)
- Days 5-7: Package Delivery
- Day 8: Replacement of Shipping Medium (liquefaction)
- Day 11: Uptake Assays

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 transport experiments is Day 11 (Monday) (see Figure 1 for details).

PreadyTake	Monday	Tuesday	Wednesday	Thursday	Friday
Week 0		**12:00 p.m. (CET) last ordering day**	*Pre-Production*		
Week 1					*Start of Production Day 1*
Week 2	*Shipment Day 4*	*Reception of Plates*			*Liquefaction Day 8*
Week 3	*Assay Performance Day 11*				

Figure 1. Timeline of manufacturing and operation for PreadyTake in 96-well format.

Equipment (not included)

- Cell culture laminar flow hood
- CO2 incubator
- Water bath
- Multichannel pipettes
- Aspiration system
- **96-well format vacuum manifold (Drummond Cat# 3-000-093 recommended)**
- Hot plate (incubator plate)
- Quantitative analytics equipment

Consumables

- Reagent reservoirs (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, Transport Buffer, Cell Lysis solutions 1 and 2 if required.*

- **HEK293 Cell Culture Medium:** Dulbecco's Modified Eagle's Medium - low glucose (SIGMA cat# D6046) supplemented with (final concentrations):
  - 10 % V/V Fetal Bovine Serum (BIOWEST Cat# DE14-801F)
  - 2 mM L-glutamine (LONZA Cat# BE17-605F)
  - 100 U/mL; 0.1 mg/mL Penicillin-Streptomycin (LONZA Cat# DE17-602F)
- **Transport Buffer solution**: Hank's 1X Balanced Salt Solutions (HBSS 1x) (HyClone Cat# SH30268) 25mM HEPES (SIGMA Cat# H7006) pH 7.4.
- **Cell Lysis Solution 1 (LS1):** 100mM NaOH
- **Cell Lysis Solution 2 (LS2):** 100mM HCl
- **Recommended reporter Substrate (stock solution)**: 10 mM Valsartan (SIGMA Cat# PHR1315) in DMSO.
- **Recommended reporter Inhibitor (stock solution)**: 10 mM Cyclosporin A (SIGMA Cat# C3662) in DMSO.

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 at 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 **90 minutes**, until the **shipping medium** reaches **liquefaction**.
3. Remove one PreadyTake plate from the incubator and place it inside the laminar flow hood.
4. Using sterile procedures (**inside the laminar flow hood**), fill a sterile reagent reservoir with 10 mL of pre-warmed (37 °C) HEK293 cell culture medium.
5. Remove all liquefied shipping medium of the PreadyTake plate by using the 96-well manifold connected to a vacuum pump (adjust aspiration flux to medium-low), taking care not to disrupt the monolayer. Make sure the shipping medium has been removed from all wells.

6. Using a multichannel pipette, dispense **100 µL** of HEK293 cell culture medium from the sterile reservoir, and fill each of the **60 wells** of the PreadyTake plate, column by column. Always add the medium against the wall of the well, and not directly onto the cell monolayers.
7. Once the shipping medium has been substituted by fresh HEK293 cell culture medium, the plates should be placed inside the incubator for **optimal recovery time (72 h)**.
8. Assay is performed on Day 11 as detailed in General Protocols for Transport Assay.

General Protocol for Transport Assays

General Considerations

PreadyTake is designed for conducting uptake transporter 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/inducers of OATP1B3.

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 OATP1B3 transport protein.

- **Reporter OATP1B3 Substrate (final concentration):** 5 µM Valsartan (SIGMA Cat# PHR1315) in transport buffer solution.
- **Reporter OATP1B3 Inhibitor (final concentration):** 1 µM Cyclosporin A (SIGMA Cat# C3662) in transport buffer solution.

Sample Plate Layout

The PreadyTake 96-well plate format allows evaluating whether a compound is a substrate and/or inhibitor of the protein transporter. Assay is performed in triplicate following the recommended plate layout shown below:

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B		Valsartan_R1	Vals/CsA_R1	Comp_C1_R1	Comp_C2_R1	Comp_C3_R1	Comp_C4_R1	Comp/CsA_C1_R1	Comp/CsA_C2_R1	Comp/CsA_C3_R1	Comp/CsA_C4_R1	
C		Valsartan_R2	Vals/CsA_R2	Comp_C1_R2	Comp_C2_R2	Comp_C3_R2	Comp_C4_R2	Comp/CsA_C1_R2	Comp/CsA_C2_R2	Comp/CsA_C3_R2	Comp/CsA_C4_R2	
D		Valsartan_R3	Vals/CsA_R3	Comp_C1_R3	Comp_C2_R3	Comp_C3_R3	Comp_C4_R3	Comp/CsA_C1_R3	Comp/CsA_C2_R3	Comp/CsA_C3_R3	Comp/CsA_C4_R3	
E		Valsartan_R1	Vals/CsA_R1	Comp_C1_R1	Comp_C2_R1	Comp_C3_R1	Comp_C4_R1	Comp/CsA_C1_R1	Comp/CsA_C2_R1	Comp/CsA_C3_R1	Comp/CsA_C4_R1	
F		Valsartan_R2	Vals/CsA_R2	Comp_C1_R2	Comp_C2_R2	Comp_C3_R2	Comp_C4_R2	Comp/CsA_C1_R2	Comp/CsA_C2_R2	Comp/CsA_C3_R2	Comp/CsA_C4_R2	
G		Valsartan_R3	Vals/CsA_R3	Comp_C1_R3	Comp_C2_R3	Comp_C3_R3	Comp_C4_R3	Comp/CsA_C1_R3	Comp/CsA_C2_R3	Comp/CsA_C3_R3	Comp/CsA_C4_R3	
H												

R: replicate
C: concentration
Comp: compound

Figure 2. Recommended sample plate layout to investigate OATP1B3-mediated transport and potential transporter-based drug-drug interactions.
Wells in light purple contain cells expressing the empty vector (HEK-MOCK or HEK-WT).
Wells in dark purple contain cells expressing the OATP1B3 transporter (HEK-OATP1B3).
Valsartan (Vals) is a OATP1B3 substrate.
Cyclosporine A (CsA) is a OATP1B3 inhibitor.

NOTE: Assay transport buffer solution should be pre-warmed to 37°C to avoid temperature stress.

NOTE: OATP1B3 substrates are assayed at 4 different concentrations. Inhibition of OATP1B3 is evaluated by incubation of the substrate in the linear range with increasing inhibitor concentrations. A further number of compounds could be assayed by using a smaller number of point dilutions.

Protocol

The following protocol applies for one plate, half seeded with OATP1B3-expressing HEK293 cells and the other half transfected with the empty vector (MOCK).

CAUTION: Do not use PreadyTake if cell monolayers do not reach at least an 80% confluency after the 72-h recovery from the shipping medium. If this is the case, take images under a phase-contrast microscope (4x magnification; 8 different wells) and contact MedTech Barcelona for replacement.

NOTE: The assay does not need to be performed under sterile conditions.

Preparation

1. **Prepare stock solutions of reference and tested compounds** in dH2O. 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. Heat an adequate amount of Transport Buffer at 37°C.
3. **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.
4. Cool down on ice the adequate amount of Transport Buffer and Cell Lysis Solutions (LS1, LS2) until further use.

Washing Steps

5. Fill a reagent reservoir with pre-warmed (37°C) Transport Buffer.
6. **Retrieve** one **"PreadyTake OATP1B3" plate from the cell incubator** and place it on the prewarmed plate incubator (hot plate).
7. Remove Maintaining Media from the wells of the "PreadyTake OATP1B3" plate by **aspiration with the 96-well manifold**. Place the manifold perpendicular to the cell monolayer and close to the insert wall.
8. **Fill** each of the 60 wells of the "PreadyTake OATP1B3" plate, column by column, with **100 µL** of pre-warmed transport buffer solution.
9. **Repeat the plate rinse** once more and **keep the plate for 15 minutes** at 37°C on the **hot plate.**

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

Transport Assay

10. **Remove the transport buffer** from the wells and **transfer 100 µL of working solutions** onto wells according to the experimental design.
11. **Incubate** the "PreadyTake OATP1B3" plate on the **hot plate** at 37°C for 30 minutes.
12. **Remove the applied working solutions** from each well immediately after the 30-min incubation to stop the assay. Immediately **rinse twice with 100 µL ice-cold transport buffer solution** with the quick adding/aspirating procedure.

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

Cell Lysis and Sample Collection

13. **Lyse cells** with 50 µL ice-cold LS1 per well.
14. **Incubate with shaking** at room temperature for 10 minutes.
15. **Neutralize LS1** with 50 µL ice-cold **LS2** per well.
16. **Transfer cell lysates** to Eppendorf tubes and **centrifuge for 5 minutes** (4ºC) at 13,000 rpm. Retrieve the required amount of supernatant for compound quantification.

Measurement

17. **Measure** the amount of the **compound transported** into the cell by an appropriate analytical method.

NOTE: Valsartan is quantified by mass spectrometry analysis.

Evaluation of Compound Permeability

Compound Net Uptake

OATP1B3-mediated transport (net uptake) is the uptake of the compound's internalization into HEK-OATP1B3 versus cells expressing the empty vector (HEK-MOCK). This value is obtained by subtracting the uptake of the cells overexpressing the empty vector (HEK-MOCK) from those overexpressing the OATP1B3 receptor (HEK-OATP1B3).

Uptake Ratio

The uptake ratio is a general measure of the involvement of active processes in compound transport. This value results from dividing the compound's uptake in the HEK293 overexpressing the OATP1B3 transporter by the uptake in those expressing the empty vector (HEK-MOCK). A compound is considered an OATP1B3 receptor substrate when the uptake ratio is above 2.

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:

- The uptake ratio of OATP1B3 substrate must be greater than 2 in those cells overexpressing the transporter.
- In the presence of an OATP1B3 inhibitor, the substrate's uptake ratio must decrease significantly (> 50 %).

HEK-OATP1B3			HEK-MOCK			Net Uptake	Uptake Ratio
Substrate/Inhibitor	Concentration (µM)	Uptake (pmols/min/10^6 cells)	Substrate/Inhibitor	Concentration (µM)	Uptake (pmols/min/10^6 cells)		
Valsartan	5	23.77 ± 10.33	**Valsartan**	5	2.25 ± 1.49	21.52 ± 8.84	11.70 ± 2.64
Valsartan/Cyclosporin A	5:1	3.62 ± 0.54	**Valsartan/Cyclosporin A**	5:1	2.48 ± 0.74	1.14 ± 1.07	1.55 ± 0.54

Table 1. Reference values for the OATP1B3 substrate (valsartan) ± an inhibitor (cyclosporin A) incubated either in HEK293 cells overexpressing the OATP1B3 transporter (HEK-OATP1B3) or in those expressing the empty vector (HEK-MOCK).
Results for Valsartan are the mean of 3 independent experiments.

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