

# Corning® TransportoCells™ Cryopreserved SLC Transporter Cells

## Frequently Asked Questions



Corning TransportoCells products are high-performance mammalian cells in a convenient, cryo-preserved format that transiently overexpress a single human SLC transporter protein. The frozen cells deliver robust data, while eliminating the time required to culture and maintain stable cell lines.

### 1. What are Corning TransportoCells products?

Corning TransportoCells products are cryopreserved HEK-293 cells transiently overexpressing human SLC drug transporter proteins. Each TransportoCells product contains an individually expressed SLC transporter.

### 2. Which human SLC transporters are available as TransportoCells products?

The SLC transporters are OATP1B1, OATP1B3, OAT1, OAT3, OCT1, OCT2, MATE1, MATE2-K, PEPT1, PEPT2, NTCP, OATP2B1, OATP1A2, OAT2, OAT4, OCTN2, OATP1B1\*5, OATP1B1\*15, and corresponding Control.

### 3. Which animal species SLC transporters are available as TransportoCells products?

Monkey Oatp1b1, dog Oatp1b4, and rat Oatp1b2.

### 4. Is any polymorphic variant of human SLC transporters offered as TransportoCells products?

Yes, two polymorphic forms of human OATP1B1, including OATP1B1\*5 and OATP1B1\*15, are offered.

### 5. Which SLC transporters are recommended for testing by the US FDA and European Medicines Agency (EMA) in the most recent guidance documents published by these regulatory agencies?

The 2017 FDA Draft Guidance recommends testing OATP1B1, OATP1B3, OAT1, OAT3, OCT1, OCT2, and MATE1, MATE2-K.

The 2013 EMA Guidance recommends testing OATP1B1, OATP1B3, OAT1, OAT3, OCT1, and OCT2. All of these transporters are available as TransportoCells products.

### 6. Is a control cell available for testing along with the TransportoCells products?

Control cells, which are parent HEK-293 cells transiently expressing empty expression vector, are available (Cat. No. 354854). We recommend that negative control cells be tested side-by-side with TransportoCells products.

### 7. Can the control cells be used as control for all TransportoCells products?

Yes, all TransportoCells products, except monkey Oatp1b1, dog Oatp1b4, and rat Oatp1b2, are subcloned in the same expression vector as the control cells. The 3 animal species Oatps are subcloned in a different vector. The background uptake activity of selected substrates in the animal species TransportoCells transfected with the new expression vector is very similar to the control cells (Cat. No. 354854). Therefore, the control cells can be used along with all TransportoCells products.

### 8. How are Corning TransportoCells products shipped and stored? Are there any special handling procedures when the product arrives in my lab?

TransportoCells products are cryopreserved cells in a 2 mL cryogenic vial. The products are shipped in a liquid nitrogen dry shipper. Upon arrival in your lab, the vials should be removed immediately from the dry shipper and placed in the vapor phase of a liquid nitrogen storage tank. The empty dry shipper should be returned following the instructions included.

**9. What assays can be performed using Corning® TransportoCells™ products?**

Typical assays that can be performed with TransportoCells products include:

- ▶ Reaction phenotyping: determining if a specific SLC transporter is capable of transporting a test compound from the outside of a cell to the inside.
- ▶ Kinetic assays: determining the  $K_m$  and  $V_{max}$  values for the uptake of a test compound.
- ▶ Inhibition profiling: determining  $IC_{50}$  and  $K_i$  values for inhibitor/drug-drug interaction (DDI) screening.

**10. What are the basic steps for preparing an uptake assay with TransportoCells products? Are detailed instructions for using TransportoCells products provided?**

The steps involved in preparing TransportoCells products for an assay include thawing and plating the cells on day one and performing the assay 24 hours after plating. Detailed instructions for thawing and plating cells, as well as conducting an uptake assay, are provided with each shipment of TransportoCells products and are available online at [www.corning.com/lifesciences](http://www.corning.com/lifesciences). The instructions can also be obtained by contacting Corning Scientific Support (ScientificSupport@corning.com).

**11. How many cells can be recovered from each vial of TransportoCells product? How many assay plates can be prepared with one vial of cells?**

One vial of TransportoCells product provides a minimum of 10 million viable cells following the thawing procedure in the Instructions for Use. Typically 10 to 15 million viable cells can be obtained per vial. One vial of cells is enough for one 24-well microplate or one 96-well microplate.

**12. What is the post-thaw viability of TransportoCells products?**

The post-thaw viability is equal to, or greater than, 80%.

**13. What is the substrate-uptake performance of TransportoCells products?**

The uptake performance of TransportoCells products is reported as the “uptake ratio.” The uptake ratio of the quality control substrate for each TransportoCells product is equal to, or greater than, 8 or 10. The uptake ratio is calculated by dividing the uptake activity of the quality control substrate in TransportoCells products by the uptake activity in the control cells.

**14. Are the products tested for mycoplasma?**

Each lot of TransportoCells products is tested and found negative for mycoplasma.

**15. Can antibiotics be used in the plating media?**

You can use antibiotics with TransportoCells products but they are not required.

**16. Is sodium butyrate recommended for use in the assay?**

Sodium butyrate is recommended to boost the uptake activity of OATP1B1, OATP1B3, MATE1, MATE2-K, PEPT1, PEPT2, and NTCP, but not OAT1, OAT2, OAT3, OAT4, OCT1, OCT2, OCTN2, OATP2B1, and animal Oatps. In-house data demonstrate that sodium butyrate has minimal effect on the uptake activity of OAT, OCT, and OATP2B1. Addition of sodium butyrate into the cell culture can negatively impact the cell confluence.

**17. What is the percentage of CO<sub>2</sub> level recommended to be used to culture the cells?**

CO<sub>2</sub> level is recommended at 5% or 8%. Our data have shown the 24 hours cell confluence and uptake activity are comparable between 5% and 8% CO<sub>2</sub> incubator. TransportoCells products data are all generated using 8% CO<sub>2</sub> incubator.

**18. During thawing and plating, when is sodium butyrate supplemented in the culture and what is the recommended concentration of sodium butyrate for each transporter?**

It is recommended to add sodium butyrate in the culture media when refeeding the cells 3 to 4 hours post plating. There is no difference observed for cell confluency and uptake activity with side-by-side comparison of supplementing sodium butyrate at the time of plating versus at the time of refeeding. Sodium butyrate concentration was optimized by titration from 1 mM to 10 mM. 2 mM sodium butyrate is recommended for use for all applicable SLC transporters due to the optimal performance for cell confluency and uptake activity.

**19. What kind of serum can be used in the plating media? Why is heat-inactivated serum not recommended?**

Non-heat-inactivated fetal bovine serum should be used to prepare the plating media. For weak adhesion cell types (i.e., HEK-293), heat-inactivated serum is not recommended, because this type of serum can affect the cells' ability to adhere to plates, leading to high variation in assay results.

**20. What surface can be used to plate Corning® TransportoCells™ products?**

Poly-D-Lysine coated plates are recommended for use with TransportoCells products.

**21. What assay buffer can be used with TransportoCells products other than HBSS (Ca<sup>2+</sup>Mg<sup>2+</sup>)?**

Krebs-Henseleit Buffer (KHB) can be used with TransportoCells products. However, KHB buffer can easily precipitate when warmed. It has to be made fresh each time before use. In-house data demonstrate HBSS without Ca<sup>2+</sup>Mg<sup>2+</sup> does not perform as well as HBSS with Ca<sup>2+</sup>Mg<sup>2+</sup>.

**22. Why is the ammonium chloride treatment required for MATE1 and MATE2-K before addition of test compounds?**

Both MATE1 and MATE2-K are localized on the apical membrane of either hepatocytes or proximal tubular cells in kidneys. They are both efflux transporters driving the translocation of cations from inside of cells to outside. The driving force of MATE1 and MATE2-K is an oppositely directed proton gradient. Pre-treatment with ammonium chloride can acidify the intracellular compartment and create the outward proton gradient which is required for MATEs-mediated uptake activity.

**23. Why is pH 6.0 uptake buffer used in PEPT1, PEPT2, and OATP1A2 cell uptake assay?**

PEPTs- and OATP1A2-mediated uptake is pH dependent requiring an inward proton gradient. The driving force of PEPTs and OATP1A2 transport is the transmembrane electrochemical proton gradient.

**24. Do test compounds need to be radiolabeled? Are non-labeled compounds and detection by mass spectrometry possible alternatives?**

Radiolabeled scintillation detection assays, nonlabeled/LCMS assays, and fluorescent detection assays have been developed using TransportoCells products. More information about using MS and fluorescent detection systems for uptake assays can be obtained by contacting Corning Scientific Support (ScientificSupport@corning.com).

**25. For detection by mass spectrometry, does the sample require any further processing such as removal of protein prior to analysis?**

The cells can be lysed with 80% acetonitrile when using mass spectrometry to facilitate protein precipitation. After centrifugation, the supernatant can be injected into an LCMS and analyzed. More information about LCMS analysis can be obtained by contacting Corning Scientific Support (ScientificSupport@corning.com)

**26. What is the expiration date of TransportoCells products when stored in liquid nitrogen vapor phase?**

Refer to the Certificate of Analysis for expiration date.

**27. What is the uptake assay “window” (i.e., how long can the cells be maintained after plating and retain optimal uptake assay performance)?**

TransportoCells products have been purposely optimized to have maximal uptake activity at 24 to 48 hours after plating. The short window of optimal uptake activity eliminates the need for cell culture maintenance and allows for quick return of assay results within 24 to 48 hours after thawing the cells.

**28. Does the assay have to be performed exactly 24 hours after plating?**

The Instructions for Use provide an optimized protocol for performing the assay at 24 hours. In-house data demonstrate that similar uptake activity can be achieved when the assays are performed between 18 and 30 hours after plating, but not longer.

**29. Can Corning® TransportoCells™ products be subcultured after the assay is complete? Can a frozen cell bank be prepared for future use?**

TransportoCells products are designed as a “consumable” for one-time use. They are not to be further cultured beyond the “best used by” time described in the instructions, which is within 48 hours after thawing and plating. By design, TransportoCells products quickly lose uptake activity 48 hours after plating.

**30. What is the organic solvent tolerance for the uptake assay using TransportoCells products? What is the recommended maximum final test compound solvent concentration?**

We recommended using less than 1% of organic solvent. In-house data demonstrate that 5% DMSO can significantly impact the uptake activity of the TransportoCells products. Other organic solvents, such as methanol and acetonitrile, have not been tested.

**31. What will cause cell peeling and how can I prevent it?**

Due to edge effect of the plate, <10% of edge peeling is normal for 24-well plate assay and 10% to 15% edge peeling is normal for 48-well plate and 96-well microplate assays. This minor peeling should not affect the assay result, especially if the assay result is normalized by total protein.

To minimize cell peeling, low or no humidity setting (i.e., remove water pan from incubator) for the incubator is recommended. In-house data demonstrate high humidity (>60%) can cause cell peeling during wash steps. However, if the dryness inside causes significant media evaporation, cell detachment will be observed. In this case, keep the humidity level in the cell culture incubator around 30% to 40% by replacing the water pan with a 100 to 200 mL beaker filled with H<sub>2</sub>O in the cell culture incubation.

Assay handling is also important in the assay. Speed controlled multi-channel pipet (with middle aspirating/dispensing speed) is recommended during the assay. If cell peeling continues to be an issue, a plating density titration experiment is recommended for the individual lab setting or use manual aspiration.

It is highly recommended to use the media components and reagents listed on the Instructions for Use guide. For example, non-heat-inactivated FBS, DMEM with high glucose without sodium pyruvate.

**32. Can TransportoCells products be used for a suspension assay?**

Yes, TransportoCells products can be used for a suspension assay with robust performance. Please contact Corning Scientific Support ([ScientificSupport@corning.com](mailto:ScientificSupport@corning.com)) for the Corning test data and protocol.

## Corning® TransportoCells™ Cryopreserved SLC Transporter Cells

Cat. No.	Description	Full Name	Gene Accession Number	Number of Cells
354851	OATP1B3/SLCO1B3	Organic aniontransporting polypeptide 1B3	NM_019844	≥10 million
354852	OCT1/SLC22A1	Organic cation transporter 1	NM_003057	≥10 million
354853	OCT2/SLC22A2	Organic cation transporter 2	NM_003058	≥10 million
354854	Vector Control	N/A	N/A	≥10 million
354855	MATE1/SLC47A1	Multidrug and toxin extrusion transporter 1	NM_018242	≥10 million
354856	MATE2-K/SLC47A2	Multidrug and toxin extrusion transporter 2K	NM_001099646	≥10 million
354857	OAT1/SLC22A6	Organic anion transporter 1	NM_004790	≥10 million
354858	OAT3/SLC22A8	Organic anion transporter 3	NM_004254	≥10 million
354859	OATP1B1*1a/SLCO1B1*1a	Organic aniontransporting polypeptide 1B1, wildtype (388A)	NM_006446.4	≥10 million
354860	PEPT1/SLC15A1	Peptide transporter 1	NM_005073	≥10 million
354861	PEPT2/SLC15A2	Peptide transporter 2	NM_021082	≥10 million
354862	OATP2B1/SLCO2B1	Organic aniontransporting polypeptide 2B1	NM_007256	≥10 million
354863	OATP1A2/SLCO1A2	Organic aniontransporting polypeptide 1A2	NM_021094	≥10 million
354864	NTCP/SLC10A1	Na+Taurocholate cotransporting polypeptide	NM_003049	≥10 million
354866	OCTN2/SLC22A5	Organic cation/carnitine transporter 2	NM_003060	≥10 million
354867	OAT2/SLC22A7	Organic anion transporter 2	NM_006672	≥10 million
354868	OAT4/SLC22A11	Organic anion transporter 4	NM_018484	≥10 million
354841	Rat Oatp1b2/Slco1b2	Rat organic anion-transporting polypeptide 1b2	NM_031650	≥10 million
354842	Dog Oatp1b4/Slco1b4	Dog organic anion-transporting polypeptide 1b4	GQ497899	≥10 million
354843	Monkey Oatp1b1/Slco1b1	Monkey organic anion-transporting polypeptide 1b1	JX866725	≥10 million
354878	OATP1B1*5/SLCO1B1*5	Organic anion-transporting polypeptide 1B1 SNP (521T>C)	NM_006446.4 with 521T>C	≥10 million
354879	OATP1B1*15/SLCO1B1*15	Organic anion-transporting polypeptide 1B1 SNPs (388A>G, 521T>C)	NM_006446.4 with 388A>G, 521T>C	≥10 million

For more specific information on claims, visit the Certificates page at [www.corning.com/lifesciences](http://www.corning.com/lifesciences).

**Warranty/Disclaimer:** Unless otherwise specified, all products are for research use only. Not intended for use in diagnostic or therapeutic procedures. Not for use in humans. Corning Life Sciences makes no claims regarding the performance of these products for clinical or diagnostic applications.

### Use of Genetically Modified Microorganisms (GMM)

Information for European Customers: These products are genetically modified as described in Corning Life Sciences technical literature. As a condition of sale, use of this product must be in accordance with all applicable local guidelines on the contained use of genetically modified microorganisms, including the Directive 2009/41/EC of the European Parliament and of the Council.

# CORNING

## Corning Incorporated Life Sciences

836 North St.  
Building 300, Suite 3401  
Tewksbury, MA 01876  
t 800.492.1110  
t 978.442.2200  
f 978.442.2476

[www.corning.com/lifesciences](http://www.corning.com/lifesciences)

### ASIA/PACIFIC

**Australia/New Zealand**  
t 61 427286832

**China**  
t 86 21 3338 4338  
f 86 21 3338 4300

**India**  
t 91 124 4604000  
f 91 124 4604099

### Japan

t 81 3-3586 1996  
f 81 3-3586 1291

### Korea

t 82 2-796-9500  
f 82 2-796-9300

### Singapore

t 65 6572-9740  
f 65 6735-2913

### Taiwan

t 886 2-2716-0338  
f 886 2-2516-7500

### EUROPE

CSEurope@corning.com

### France

t 0800 916 882  
f 0800 918 636

### Germany

t 0800 101 1153  
f 0800 101 2427

### The Netherlands

t 020 655 79 28  
f 020 659 76 73

### United Kingdom

t 0800 376 8660  
f 0800 279 1117

### All Other European Countries

t +31 (0) 206 59 60 51  
f +31 (0) 206 59 76 73

### LATIN AMERICA

grupoLA@corning.com

### Brasil

t 55 (11) 3089-7400

### Mexico

t (52-81) 8158-8400

For additional product or technical information, visit [www.corning.com/lifesciences](http://www.corning.com/lifesciences) or call 800.492.1110. Outside the United States, call +1.978.442.2200 or contact your local Corning sales office.