

Corning® TransportoCells™

SLC Transporter Cells

Frequently Asked Questions

CORNING

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 HEK293 cells transiently overexpressing human SLC drug transporter proteins. Each TransportoCells product contains an individually expressed SLC transporter.

2. Which SLC transporters are available as TransportoCells products?

The SLC transporters are OATP1B1, OATP1B3, OAT1, OAT3, OCT1 and OCT2.

3. Which SLC transporters are recommended for testing by the FDA and EMA in the most recent guidance documents published by these regulatory agencies?

The 2012 FDA Draft Guidance recommends testing OATP1B1, OATP1B3, OAT1, OAT3 and OCT2. The 2013 EMA Guidance recommends testing OATP1B1, OATP1B3, OAT1, OAT3, OCT1 and OCT2. All of these transporters are available as TransportoCells products.

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

Control cells, which are parent HEK293 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.

5. How are TransportoCells products shipped and stored? Are there any special handling procedures when the product arrives at my lab?

TransportoCells products are cryopreserved cells in a 2 mL cryogenic vial. The product is 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.

6. What assays can be performed using 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.

7. 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 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.

**8. How many cells can be recovered from each vial of Corning® 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 are enough for one 24 well plate or one 96 well plate.

9. What is post-thaw viability of TransportoCells products?

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

10. What is 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, 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.

11. Are the products tested for mycoplasma?

TransportoCells products are tested and found negative for mycoplasma.

12. Can antibiotics be used in the plating media?

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

13. Is sodium butyrate recommended for use in the assay?

Sodium butyrate is recommended to boost the uptake activity of OATP1B1 and OATP1B3, but not OAT1, OAT3, OCT1 and OCT2. In-house data demonstrate that sodium butyrate has minimal effect on the uptake activity of OATs and OCTs. Addition of sodium butyrate into the cell culture can negatively impact the cell confluence.

14. What surface can be used to plate TransportoCells products?

Poly-D-Lysine coated plates are recommended for use with TransportoCells products. Other surfaces can be used, but the cell attachment, confluency and assay robustness might be compromised.

15. What assay buffer can be used with TransportoCells products other than HBSS (Ca²⁺Mg²⁺)?

Krebs-Henseleit Buffer (KHB) can be used with TransportoCells products. However, KHB buffer can easily form precipitation when warmed. It has to be made fresh each time before use. In-house data demonstrate HBSS without Ca²⁺Mg²⁺ does not perform as well as HBSS with Ca²⁺Mg²⁺.

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

Radiolabeled scintillation detection assays, non-labeled/LC-MS 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 Technical Support at CLStechserv@corning.com.

17. For detection by mass spectrometry, does the sample require any further process, 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 LC-MS and analyzed. More information about LC-MS analysis can be obtained by contacting Corning Technical Support at CLStechserv@corning.com.

18. How long can TransportoCells products be kept in liquid nitrogen vapor phase storage?

TransportoCells products have a 3-year shelf life.

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

Corning® 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.

20. 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 hours and 30 hours after plating, but not longer.

21. Can 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.

22. 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.

Corning® TransportoCells™ Cryopreserved SLC Transporter Cells Ordering Information

Cat. No.	Description	Full Name	Gene Accession Number	Quantity
354859	OATP1B1*1a/SLCO1B1*1a	Organic anion-transporting polypeptide 1B1 Wild Type (388A)	NM_006446.4	10 million cells
354851	OATP1B3/SLCO1B3	Organic anion-transporting polypeptide 1B3	NM_019844	10 million cells
354857	OAT1/SLC22A6	Organic anion transporter 1	NM_004790	10 million cells
354858	OAT3/SLC22A8	Organic anion transporter 3	NM_004254	10 million cells
354852	OCT1/SLC22A1	Organic cation transporter 1	NM_003057	10 million cells
354853	OCT2/SLC22A2	Organic cation transporter 2	NM_003058	10 million cells
354854	Control			10 million cells

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

Worldwide Support Offices

ASIA/PACIFIC
Australia/New Zealand
t 0402-794-347

China
t 86 21 2215 2888
f 86 21 6215 2988

India
t 91 124 4604000
f 91 124 4604099

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

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Singapore
t 65 6733-6511
f 65 6861-2913

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

EUROPE

France
t 0800 916 882
f 0800 918 636

Germany
t 0800 101 1153
f 0800 101 2427

The Netherlands
t 31 20 655 79 28
f 31 20 659 76 73

United Kingdom
t 0800 376 8660
f 0800 279 1117

All Other European Countries

t 31 (0) 20 659 60 51
f 31 (0) 20 659 76 73

LATIN AMERICA Brasil

t (55-11) 3089-7419
f (55-11) 3167-0700

Mexico
t (52-81) 8158-8400
f (52-81) 8313-8589

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