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Corning® TransportoCells™ HEK293-derived Human MRP2 Vesicles

Catalog Number.....453803
Lot Number.....6342018

Storage Conditions.. -80°C
Date Released.....2016 December
Expiration Date.....2021 December

Product Description: MRP2 membrane vesicles are prepared from Human Embryonic Kidney (HEK) 293 cells overexpressing human multidrug resistance-associated protein 2 (MRP2, ABCC2) (Gene Accession No. NM_000392). MRP2 is an efflux transporter and expressed primarily in liver, kidney, gastrointestinal tract (GI), and placenta. In liver, MRP2 is important for elimination of organic anions (drug conjugates and conjugated bilirubin) into bile. It also plays a similar role in renal elimination in kidney.

Negative Control Vesicles (Cat. No. 453800) prepared from HEK293 cells transfected with empty vector can be used as a control for this product.

Package Contents.....2.5 mg protein in 0.5 mL
Protein Content.....5.0 mg/mL in TMEP*

*TMEP: 50 mM Tris-Base, 50 mM Mannitol, 2 mM EGTA, 2 mM 2-Mercaptoethanol, pH 7.0 and a 1:1000 dilution of Protease Inhibitor Cocktail (Sigma Cat. No. P-8340).

QUALITY CONTROL:

Specification	Criteria		Result
Uptake Assay	Transporter:	Human MRP2	PASS
	Substrate:	50 µM estradiol-17β-glucuronide	
	Incubation time:	5 min	
	S/N Ratio*:	≥10	

*S/N Ratio: Signal to noise ratio is the ratio of uptake activity with ATP versus uptake activity with AMP.

METHOD: Corning Gentest™ MRP/BCRP Vesicle Assay Kit (Cat. No. 459010) is used to perform the uptake assay of MRP2 vesicles. The activity is measured using a rapid filtration technique. A 60 µL reaction mixture containing 50 µg vesicles and 50.0 µM E17βG in Assay Uptake Buffer (47 mM MOPs, 65 mM KCl, 7 mM MgCl2, pH 7.4) is pre- incubated at 37°C for 10 min. Uptake transport is initiated by addition of 15 µl of 25 mM MgATP or 25 mM AMP into the samples followed by 5 min. incubation at 37°C. The reaction is terminated by transferring the vesicles/substrate mixture to a glass fiber filter plate followed by washing with 1X cold wash buffer (40 mM MOPs, 70 mM KCl, pH 7.4) for five times. After the filter plate is completely dry, the radioactivity is measured with scintillation counter.

STORAGE and HANDLING

- Thaw vesicles on ice and keep on ice until use.
- The vesicle activity can be compromised upon freezing and thawing. After initial thaw, it is highly recommended to aliquot into single use vials to minimize freeze-thaw cycles.
- Triplicate samples per assay conditions are recommended.

For Research Use Only. Not for use in diagnostic or therapeutic procedures.

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- The concentration of test compound and incubation time usually depends upon the specific characteristics of the drug.

Quality Assurance: _____

Date: March 23, 2017

SAFETY RECOMMENDATIONS:
Safety assessment indicates this product is not hazardous, therefore no SDS (Safety Data Sheet) is provided. Handle in accordance with good industrial hygiene and laboratory safety practices.

Corning® TransportoCells™ HEK293-derived ABC Transporter Membrane Vesicles

Instructions for Use

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ATP-binding cassette (ABC) transporters are multi-transmembrane-bound proteins primarily expressed in the tissues involved in drug absorption, distribution and elimination, (i.e., intestine, liver, kidney, and blood brain barrier). Drug-drug interaction occurring at the site of ABC transporter functions can lead to alteration of drug pharmacokinetics and/or toxicity. Current regulatory guidance includes two ABC transporters, MDR1/P-gp and BCRP, in the recommended list of important transporters for drug-drug interaction (DDI) tests. In light of the evidence showing the importance of MRP2 and BSEP in drug-induced liver injury, it is important to evaluate the potential interaction of new molecular entities (NMEs) with MRP2 and BSEP. Due to the physical localization of the efflux transporter on the plasma membrane and the physicochemical properties of substrates, inside-out membrane vesicles are a suitable *in vitro* model for the direct determination of ABC transporter substrates or inhibitors.

Corning® TransportoCells™ ABC transporter vesicles are membrane-bound vesicles made from mammalian cells (HEK-293) which transiently over-express a single human ABC transporter protein. Substrate phenotyping and inhibition studies for DDI are easily carried out using a variety of detection systems including scintillation counting for radiolabeled compounds, mass spectrometry for non-radiolabeled compounds, and fluorescence detection for fluorescent compounds. The product portfolio includes four human ABC transporter vesicles and a control vesicle, as listed in the table below.

This Instructions for Use document describes the procedure for performing an uptake assay with the Corning TransportoCells ABC transporter vesicles.

PRODUCTS

Cat. No.	Description	Full Name	Gene Accession No.
453800	Control vesicle	HEK293-derived negative control vesicle	N/A
453801	Human MDR1/P-gp vesicles	HEK293-derived Human MDR1/P-gp vesicles	NM_000927
453802	Human BSEP vesicles	HEK293-derived human BSEP vesicles	NM_003742
453803	Human MRP2 vesicles	HEK293-derived human MRP2 vesicles	NM_000392
453804	Human BCRP vesicles	HEK293-derived human BCRP vesicles	NM_004827
459010	Assay reagent kit	MRP/BCRP vesicle assay kit	N/A
459011	Assay reagent kit	BSEP vesicle assay kit	N/A

REAGENTS AND DISPOSABLES

Reagent/Disposable	Supplier	Supplier Cat. No.
Dimethyl Sulfoxide (DMSO)	MilliporeSigma	D2438
Reagent reservoirs	VWR	82026-350
1.7 mL microcentrifuge tubes	VWR	53550-698
0.1N Sodium hydroxide (NaOH)	J.T. Baker	5636-02
0.1N H_2SO_4	MilliporeSigma	319589
SDS	MilliporeSigma	L3771
[3H] Estradiol-17β-D-glucuronide	PerkinElmer	NET1106
[3H] Estrone-3-sulfate	PerkinElmer	NET203
[3H] Taurocholic acid	PerkinElmer	NET322
N-methyl-quinidine	MilliporeSigma	SBNMQ
96-well black flat-bottom assay microplate	Corning	3915
96-well clear assay microplate	Corning	353075
96-well glass fiber (G/F) filter microplate	PerkinElmer	6005177
96-well glass fiber (G/F) filter microplate	MilliporeSigma	MSFBN6B10
Plate seals	PerkinElmer	6005185
Betaplate scintillation liquid	PerkinElmer	1205-440

MATERIALS AND EQUIPMENT

- LC-MS/MS
- Centrifuge
- Ice bucket
- 37°C incubator
- Water bath
- Fluorescence microplate reader
- Cell harvester or vacuum manifold
- Scintillation counter or equivalent
- Multichannel pipettor and corresponding tips
- Pipets: 10, 20, 200, 1,000 µL pipets and corresponding tips
- Deionized water

ASSAY PROCEDURE

Before starting:

- Read the entire protocol before starting an assay.
- Please refer to the Instructions for Use for Reagent Kit (see below) for performing the assay.
  - **MRP/BCRP vesicle uptake assay kit** (Corning Cat. No. 459010) is designed for MDR1/P-gp, MRP2, and BCRP vesicle uptake assay.
  - **BSEP vesicle uptake assay kit** (Corning Cat. No. 459011) is designed for BSEP vesicle uptake assay.
- The following procedure describes the vesicle uptake assays using the probe substrate as listed below: N-methyl-quinidine (NMQ) for MDR1/P-gp; estradiol-17β-D-glucuronide (E17βG) and 5(6)-Carboxy-2,'7'-Dichlorofluorescein (CDCF) for MRP2; estrone-3-Sulfate (E3S) for BCRP; and taurocholic acid (TCA) for BSEP.
- The recommended apparatuses for radiolabeled, non-radiolabeled compound, and fluorescence compound are listed in the following table:

	Rapid Filtration System	GF/B Filter Plate	Collection Plate
Radiolabeled Compounds	Cell Harvester (PerkinElmer or equivalent)	96-well glass fiber (G/F) filter microplate (PerkinElmer Cat. No. 6005177 or equivalent)	N/A
	Vacuum Manifold (MilliporeSigma Cat. No. MSVMHTS00 or equivalent)	96-well glass fiber filter microplate (MilliporeSigma Cat. No. MSFBN6B10 or equivalent)	N/A
Non-radiolabeled Compounds	Vacuum Manifold (MilliporeSigma Cat. No. MSVMHTS00 or equivalent)	96-well glass fiber filter microplate (MilliporeSigma Cat. No. MSFBN6B10 or equivalent)	96-well clear assay microplate (Corning Cat. No. 353075)
Fluorescence Substrate			96-well black flat-bottom assay microplate (Corning Cat. No. 3915)

I. Uptake Assay for Radiolabeled Compounds

Refer to the following table for recommended positive control substrate concentration, incubation time, and buffers:

Corning Cat. No.	Description	Substrate	Incubation Time (min.)	Buffer
453803	Human MRP2 vesicles	50 µM E17βG	5	MOPs
453804	Human BCRP vesicles	1 µM E3S	2	MOPs
453802	Human BSEP vesicles	5 µM TCA	5	HEPES

A. Preparation of Vesicle/Substrate Mix

1. Dissolve test compound at desired concentration in DMSO or other solvent. The stock concentration of test compound should be at least 50 times the final testing concentration (the final concentration of solvent should remain less than 2%).

2. Prepare appropriate positive control substrate solutions for the desired transporter in the following tables.

   NOTE: The assay is recommended to be performed in triplicate.

   ***NOTE:** The stock concentration of radiolabeled compound may vary batch-to-batch.

   - Prepare master mix of positive control substrate or test compound at 1.25X desired final concentration.
   - The vesicle/test compound mix can be prepared based on the tested concentration accordingly.

   MRP2 Vesicles

Description	Concentration (1.25X)	Volume (µL)
20 mM E17βG	62.3 µM	1.3
*20 µM [3H]-E17βG	0.2 µM	4.2
300 mM GSH	2.5 mM	3.5
MOPs vesicle uptake buffer	–	341.0

   BCRP Vesicles

   Prepare 100 µM E3S working solution by diluting 1 mM stock in uptake buffer.

Description	Concentration (1.25X)	Volume (µL)
100 µM E3S	1.2375 µM	5.2
*2.19 µM [3H]-E3S	0.0125 µM	2.4
MOPs vesicle uptake buffer	–	342

   BSEP Vesicles

   Prepare 100 µM TCA working solution by diluting 1 mM stock in uptake buffer.

Description	Concentration (1.25X)	Volume (µL)
100 µM TCA	0.625 µM	2.6
*200 µM [3H] TCA	0.625 µM	1.3
HEPES vesicle uptake buffer	–	346

3. Add 70 µL of vesicles (5 mg/mL) to the appropriate Eppendorf tube containing the substrate mixture prepared in the above step. **NOTE:** The final vesicle concentration is 0.05 mg/well.
4. Mix solution gently by pipetting up and down. DO NOT VORTEX. Aliquot 60 µL of the mixture to the appropriate wells into the 96-well microplate.

   NOTE: Save the rest of vesicle/substrate mixture for normalization when performing data analysis.

5. Prepare the ATP and AMP working solutions:

	200 mM ATP or AMP stock	Assay Uptake Buffer
25 mM ATP working solution	125 µL	875 µL
25 mM AMP working solution	125 µL	875 µL

B. Reaction Initiation and Termination

NOTE: For TCA uptake assay in BSEP vesicles, the glass fiber filter microplate needs to be pre-conditioned with 0.1% BSA to block the non-specific binding (steps 3 and 4).

1. Pre-incubate the 96-well microplate containing vesicle/substrate mixture at 37°C for 10 min. Pre-incubate the 25 mM ATP and the 25 mM AMP working solutions at 37°C for 10 min.
2. During the period of pre-incubation, prepare the glass fiber filter microplate by adding 200 µL of water or uptake buffer into each well and filtering.
3. For BSEP only, prepare 1X blocking buffer (0.1% BSA) by diluting 50X blocking buffer with HEPES wash buffer. Add 200 µL the blocking buffer to each of the appropriate wells on the filter microplate. Filter and wash the filter microplate with pre-chilled 1X wash buffer five times.
4. After the pre-incubation, aliquot 15 µL of 25 mM ATP or 15 µL of 25 mM AMP into the appropriate wells using the multichannel pipettor.
5. Incubate the microplate at 37°C and according to the table in Section 1.
6. At the end of incubation, the reaction is terminated by adding pre-chilled 1X wash buffer when using a cell harvester. When using a vacuum manifold, stop the reaction by adding 200 µL of pre-chilled 1X wash buffer using a multichannel pipettor, then transfer the reaction mix to a filter microplate using a multichannel pipettor. Apply vacuum.

7. Wash the filter mciroplate with pre-chilled 1X wash buffer five times. Apply vacuum for an extra 15 seconds after the last wash.
8. Dry the filter microplate two to three hours at room temperature. If a fan is used to blow dry the filter miroplate, the time can be reduced to approximately one hour.
9. After the filter microplate is dry, seal the bottom of the microplate. Add 10 µL of remaining vesicle/ substrate mixture prepared in Step A2 to three empty wells on the same filter microplate to be used for signal normalization.
10. Add 50 µL of betaplate scintillation fluid to the appropriate wells on filter microplate, then seal the top of the microplate.
11. Measure the radioactivity using a scintillation counter.
12. Refer to Section III(A) for analysis of results.

For non-radiolabeled compounds for LC-MS/MS analysis, following Steps 13 through 19.

13. During the period of drying, prepare substrate standard curve in the same elution buffer used in following elution steps.
14. After the microplate is dry, place the filter microplate onto a 96-well receiver microplate so that it collects the flow through.
15. Add 50 µL of elution buffer containing the appropriate analytical internal standard at defined concentration to each of the assay wells.

    NOTE: Elution buffer can be either 80% acetonitrile or 80% methanol.
16. Centrifuge the filter microplate with the collection microplate at 2,000 rpm for 5 minutes.
17. After the centrifugation, add an additional 50 µL of elution buffer containing the analytical internal standard at the defined concentration to each of the assay wells; and add 100 µL of each standard curve sample into the appropriate well.
18. Repeat Step 16.
19. Analyze the samples on LC-MS/MS with the appropriate developed assay parameters for each of the specific substrate and internal standard compounds.

II. Uptake Assay for Fluorescent Compounds

Refer to the following table for recommended positive control substrate concentration, incubation time, buffers, elution reagents, and wavelength:

Corning Cat. No.	Description	Substrate	Incubation Time (min.)	Buffer	Elution Reagent(s)	Wavelength
453801	Human MDR1 vesicles	5 µM NMQ	2	MOPs	10% SDS and 0.1N H_2SO_4	Ex.: 355 nm Em.: 460 nm
453803	Human MRP2 vesicles	5 µM CDCF	5	MOPs	0.1 N NaOH	Ex.: 485 nm Em.: 538 nm

A. Preparation of Vesicle/Substrate Mix

1. Prepare appropriate positive control substrate solutions for the desired transporter in the following tables.

   NOTES:

   - It is recommended that the assay be performed in triplicate.
   - Prepare master mix of the fluorescent substrate at 1.25X desired final concentration.

   MDR1 Vesicles

   Prepare 10 mM NMQ stock in DMSO. Next, prepare 1 mM NMQ working solution by diluting 10 mM stock in uptake buffer.

Description	Concentration (1.25X)	Volume (µL)
1 mM NMQ	6.25 µM	2.6
MOPs vesicle uptake buffer	–	347.4

   MRP2 Vesicles

Description	Concentration (1.25X)	Volume (µL)
1 mM CDCF	6.25 µM	2.6
300 mM GSH	2.5 mM	3.5
MOPs vesicle uptake buffer	–	344

2. Add 70 µL of vesicles (5 mg/mL) to the appropriate microcentrifuge tube containing the substrate mixture prepared in the above step. **NOTE:** The final vesicle concentration is 0.05 mg/well.
3. Mix solution gently by pipetting up and down. DO NOT VORTEX. Aliquot 60 µL of the mixture to the appropriate wells into the 96-well microplate.
4. Prepare the ATP and AMP working solutions:

	200 mM ATP or AMP Stock	Assay Uptake Buffer
25 mM ATP working solution	125 µL	875 µL
25 mM AMP working solution	125 µL	875 µL

B. Reaction Initiation and Termination

5. Pre-incubate the 96-well microplate containing vesicle/substrate mixture at 37°C for 10 min. Pre-incubate the 25 mM ATP and the 25 mM AMP working solutions at 37°C for 10 min.
6. During the period of pre-incubation, prepare the glass fiber filter microplate by adding 200 µL of water or uptake buffer into each well and filtering.
7. After pre-incubation, aliquot 15 µL of 25 mM ATP or 15 µL of 25 mM AMP into the appropriate wells using the multichannel pipettor, respectively.
8. Incubate the microplate at 37°C and according to the table in Section II.
9. At the end of incubation, terminate the reaction by placing the 96-well reaction microplate on ice and immediately adding pre-chilled 1X wash buffer using a multichannel pipettor. Then transfer the reaction mix to a filter microplate using a multichannel pipettor and apply vacuum.
10. Wash the filter microplate with pre-chilled 1X wash buffer five times. Apply vacuum for an extra 15 seconds after the last wash.
11. Leave the filter microplate in the dark and dry for 2 to 3 hours at room temperature. If a fan is used to blow dry the filter microplate, the time can be reduced to approximately one hour.

C. Preparation of Standard Curve and Elution

1. During the period of drying, prepare the substrate standard curve according to the specific substrate directions below.
2. After the microplate is dry, prepare the filter microplate onto a 96-well black flat-bottom microplate so that it collects the flow through.

For NMQ for MDR1 vesicles, refer to Steps 3 through 9.

3. NMQ standard curve: Prepare the highest concentration at 4 µM by diluting the NMQ stock in 10% SDS followed by 1:1 dilution in 10% SDS. The standard curve ranges from 4 µM to 0.03125 µM.
4. Add 50 µL of 10% SDS to each of the assay wells.
5. Centrifuge the filter microplate with the collection microplate at 2,000 rpm for 5 minutes.
6. After centrifugation, add an additional 50 µL of 10% SDS to each assay well, and add 100 µL of each standard curve sample into the appropriate well; add 100 µL of 10% SDS into the assay wells serving as the blank.
7. Repeat Step 5.
8. Remove the filter microplate from the collection microplate. Add 100 µL of 0.1N H_2SO_4 into each of the assay wells, standard curve wells, and the blank well on the collection microplate.
9. Measure the fluorescence intensity at wavelength of Ex. 355 nm and Em. 460 nm on a fluorescence reader. Refer to Section III(B) for analysis of results.

For CDCF for MRP2 vesicles, refer to Steps 10 through 15.

10. CDCF standard curve: Prepare the highest concentration at 800 nM by diluting the CDCF stock in 0.1N NaOH followed by 1:1 dilution in 0.1N NaOH. The standard curve ranges from 800 nM to 25 nM.
11. Add 50 µL of 0.1N NaOH to each of the assay wells.
12. Centrifuge the filter microplate with the collection microplate at 2,000 rpm for 5 minutes.
13. After centrifugation, add an additional 50 µL of 0.1N NaOH to each assay well, and add 100 µL of each standard curve sample into the appropriate well; add 100 µL of 0.1N NaOH to the assay wells serving as the blank.
14. Repeat Step 12.

15. Remove the filter microplate from the collection microplate.
16. Measure the fluorescence intensity at wavelength of Ex. 485 nm and Em. 538 nm on a fluorescence reader. Refer to Section III(B) for analysis of results.

III. Analysis of Results

A. For Radiolabeled Compounds:

1. Determine the activity (CCPM/pmol) by performing the following calculation:

$$\text{CCPM/pmol} = \frac{\text{Total CCPM of Normalization Samples}}{(\text{Total Volume } \mu\text{L for Normalization}) \times (\text{Concentration of Normalization Solution } \mu\text{M})}$$

2. Calculate the uptake activity with ATP and AMP using the following equations:

$$\text{Uptake activity with ATP (pmol/min/mg)} = \frac{\text{Average CCPM of Duplicate or Triplicate with ATP}}{(\text{CCPM/pmol}) \times \text{Incubation Time (min.)} \times \text{Amount of Protein (mg)}}$$

$$\text{Uptake activity with AMP (pmol/min/mg)} = \frac{\text{Average CCPM of Duplicate or Triplicate with AMP}}{(\text{CCPM/pmol}) \times \text{Incubation Time (min.)} \times \text{Amount of Protein (mg)}}$$

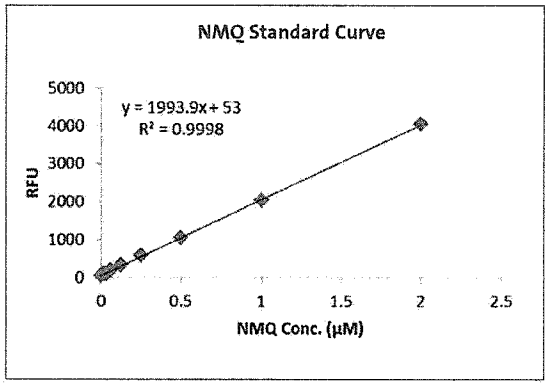
$$\text{ATP-dependent uptake activity} = \text{Uptake activity with ATP (pmol/min./mg) - Uptake activity with AMP (pmol/min./mg)}$$

$$\text{Signal-to-Noise (S/N) Ratio} = \text{Uptake activity with ATP (pmol/min./mg)/Uptake activity with AMP (pmol/min./mg)}$$

B. For Fluorescence Compounds:

1. Analyze the standard curve by linear regression to determine the slope and intercept. Example follows:

NMQ	Concentration (μM)	RFU
STD 1	2	4,048
STD 2	1	2,026
STD 3	0.5	1,041
STD 4	0.25	585
STD 5	0.125	324
STD 6	0.0625	180
STD 7	0.03125	107
STD 8	0.015625	89
Blank	0	47
Slope	1,993.9	–
Intercept	53	–
R2	0.9998	–



2. Calculate the concentration of each sample using the slope and intercept determined above.
3. Calculate the uptake activity with ATP and AMP using the following equations:

$$\text{Uptake activity with ATP (pmol/min./mg)} = \frac{\text{Sample Concentration (}\mu\text{M)} \times \text{Final Elute Volume (}\mu\text{L)}}{\text{Incubation Time (min.)} \times \text{Amount of Protein (mg)}}$$

$$\text{Uptake activity with AMP (pmol/min./mg)} = \frac{\text{Sample Concentration (}\mu\text{M)} \times \text{Final Elute Volume (}\mu\text{L)}}{\text{Incubation Time (min.)} \times \text{Amount of Protein (mg)}}$$

$$\text{ATP-dependent uptake activity} = \text{Uptake activity with ATP (pmol/min/mg) - Uptake activity with AMP (pmol/min./mg)}$$

$$\text{Signal-to-Noise (S/N) Ratio} = \frac{\text{Uptake activity with ATP (pmol/min./mg)}}{\text{Uptake activity with AMP (pmol/min./mg)}}$$

SAFETY RECOMMENDATIONS

Safety assessment indicates this product is not hazardous; therefore, no SDS (Safety Data Sheet) is provided. All reagents should be handled in accordance with good industrial hygiene and laboratory safety practices.

For more specific information on claims, visit the Certificates page at **www.corning.com/lifesciences**.

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