Corning® TransportoCells™ HEK293-derived ABC Transporter Vesicles

CORNING

ATP-binding cassette (ABC) transporters are an important piece of drug efflux and have a significant impact on drug pharmacokinetics, efficacy, and safety. BCRP and P-gp are two ABC transporters that current regulatory guidance has identified as important transporters to test for drug-drug interactions. Evidence identifying the importance of BSEP and MRP2 in drug-induced liver injury has prompted the regulatory agencies to expand the recommended evaluation of new molecular entities to include BSEP and MRP2 in the next revision. Recent research showed that another two efflux transporters MRP3 and MRP4 may play a compensatory role for predicting bile acid associated hepatotoxicity. Efflux transporters are localized on the plasma membrane. This localization, combined with the physicochemical properties of substrates, have made inside-out membrane vesicles the standard for studying drug interactions with ABC transporters.

Commonly used membrane vesicles are made using Sf9 cells infected with baculovirus. The disadvantages associated with this system include: different membrane composition than mammalian cells, different protein post-translation modification leading to different function, and lack of consistency. Corning has developed Corning TransportoCells HEK293-derived ABC transporter vesicles, which are produced using a mammalian cell expression system.

Corning's HEK293-derived vesicles express more human-like transporter proteins within the human cellular membrane. These HEK membrane vesicles have demonstrated a significantly higher uptake activity and lower background as compared with insect cell-derived vesicles. The validated vesicle model could potentially improve *in vitro* to *in vivo* prediction because of the more *in vivo*-like recombinant proteins and membrane composition.

Features and Benefits of Corning TransportoCells Vesicle Models

Mammalian Cell Expression System

Corning TransportoCells Vesicle models contain more *in vivo*-like recombinant proteins and membrane composition, thus providing the potential for a better *in vitro* to *in vivo* correlation.

Reliable and Robust

Corning TransportoCells Vesicles have been demonstrated to have a robust dynamic range with significantly higher uptake activity and lower background than insect cell expression systems.

The model has been fully validated for substrate specificity, transporter kinetics, and inhibition profiles to ensure data are consistent with existing vesicle models.

Supports Regulatory Recommendations

Corning TransportoCells Vesicle models support the USFDA, EMA, and ITC recommendations for the identification of drug transporters and transporter drug-drug interaction studies critical in the development of new investigational drugs.

Reduce Biosafety Concerns

Corning TransportoCells ABC vesicles have been engineered in HEK293 cells, without the need for baculovirus, thus eliminating the safety concerns.

Applications

- Drug transporter phenotyping
- Drug-drug interaction and toxicity studies
- Drug clearance prediction
- Mechanistic studies of drug transport

Uptake Activity Performance

Table 1. Summary of the performance of HEK293-derived ABC transporter vesicles. Uptake activity of MDR1/P-gp, BSEP, MRP2, and BCRP vesicles in the presence of ATP or AMP were evaluated by incubating the vesicles with the listed prototypical substrates at the indicated concentration for the period of time within linear range. Uptake ratio is calculated by dividing uptake activity in the presence of ATP by activity in the presence of AMP.

Transporters	Probe Substrate	Probe Substrate Conc. (µM)	Incubation Time (min.)	Uptake with ATP (pmol/mg/min.)	Uptake with AMP (pmol/mg/min.)	Signal-to-Noise Ratio (S/N)
Human MDR1/P-gp	N-methyl-quinidine	5	2	2,762	62	45
Human BSEP	Taurocholic Acid	1	5	78	3.1	25
Human MRP2	Estradiol-17β-glucuronide	50	5	3,948	58	68
Human BCRP	Estrone-3-sulfate	1	2	60.4	4.1	15
Human MRP3	Estradiol-17β-glucuronide	1	5	49.3	4.4	11
Human MRP4	Dehydroepiandrosterone 3-sulfate	2	5	49.9	4.3	12

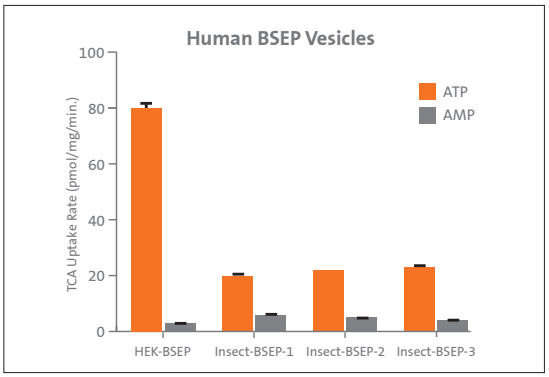

Figure 1. Comparison of uptake dynamics of ABC transporter vesicles derived from mammalian cells vs. insect cells. ATP-dependent uptake of 1 µM taurocholic acid (TCA) and signal-to-noise ratio was significantly higher in HEK293-BSEP vesicles, compared to the BSEP vesicles made from insect cell-based expression systems from three commercial sources.

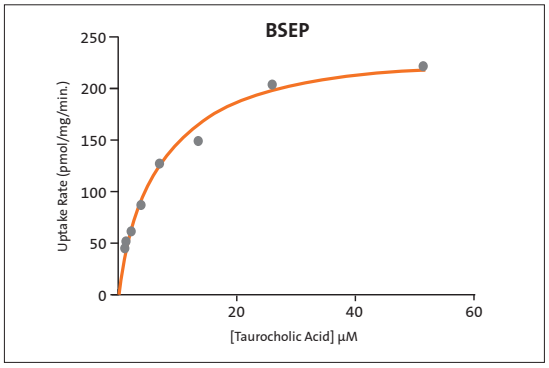

Figure 2. Kinetic assay. The solid line represents the nonlinear regression of ATP-dependent uptake activity, which was calculated using uptake activity in the presence of ATP minus uptake activity in the presence of AMP. K_m and V_{max} values for the listed ABC transporters are comparable to those reported in the literature or Corning internal data generated using insect cell-derived vesicles.

Corning® HEK293 ABC Transporter Membrane Vesicles

		Generated Data		Reference Data	
Transporter	Substrate	K_m (µM)	V_{max} (pmol/mg/min.)	K_m (µM)	V_{max} (pmol/mg/min.)
Human MDR1/P-gp	NMQ	4.9	4,624	3.65[a]	656[a]
Human BSEP	TCA	6.3	241	8.4[b]	20.1[b]
Human MRP2	E17βG	124	5,751	148[b]	1,203[b]
Human MRP3	E17βG	4.0	116	9.1[c]	116[c]
Human MRP4	DHEAS	4.0	61	3.5[c]	387[c]
Human BCRP	E3S	7.1	272	14.2[b]	506[b]

[a]Herédi-Szabó K, et al. Eur J Pharm Sci. 2013, 49(4):773-81.
[b]Data generated using insect cells-derived membrane vesicles by Corning Life Sciences.
[c] Kock K, et al. Drug Metab Dispos. 2014, 42:665-674.

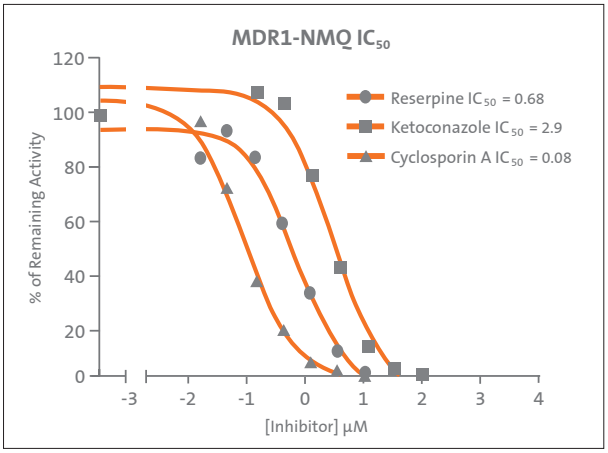

Figure 3. Inhibition assay. IC_{50} values were generated by co-incubating vesicles with probe substrate in the presence of inhibitors at a range of concentrations with ATP or AMP. The data represent the percentage of remaining ATP-dependent uptake activity. IC_{50} values (unit: µM) are comparable to those published in the literature or Corning internal data generated using insect cell-derived vesicles.

Corning HEK293-derived ABC Transporter Membrane Vesicles

Transporter	Substrate	Inhibitor	Generated Data IC_{50} (µM)	Reference Data IC_{50} (µM)
Human MDR1/P-gp	NMQ	Ketoconazole	2.9	3.77[a]
		Cyclosporin A	0.08	0.147[a]
		Reserpine	0.68	0.62[a]
Human BSEP	TCA	Troglitazone	2.4	2.7[b]
		Glibenclamide	1.7	5.3[b]
		Ketoconazole	1.1	2.9[b]
Human MRP2	CDCF	MK571	2.5	6.2[c]
		Terfenadine	103	45.8[c]
		Indomethacin	41	90.8[c]
Human MRP3	E17βG	MK571	9.8	20.3[c]
		Fidaxomicin	0.58	1.3[c]
		Furosemide	108	252[c]
Human MRP4	DHEAS	MK571	2.1	10[d]
		Indomethicin	5.8	6.1[d]
		Quercetin	1.6	1.2[d]
Human BCRP	E3S	Sulfasalazine	0.42	0.7[c]
		Fumitremorgin C	0.13	0.1[c]
		Nobomycin	0.1	0.6[c]

[a] Herédi-Szabó K, et al. Eur J Pharm Sci., 2013, 49(4):773-81.t
[b] Dawson S, et al. Drug Metab Dispos. 2012, 40(1):130-8.
[c] Data generated using insect cell-derived membrane vesicles by Corning Life Sciences.
[d] Wen J, et al. J Pharmacol Exp Ther, 2015, 354:358-75.

Ordering Information

Cat. No.	Description	Gene Accession No.	Protein Concentration	Qty
453800	HEK293-derived negative control vesicle	N/A	5 mg/mL	0.5 mL
453801	HEK293-derived Human MDR1/P-gp vesicles	NM_000927	5 mg/mL	0.5 mL
453802	HEK293-derived human BSEP vesicles	NM_003742	5 mg/mL	0.5 mL
453803	HEK293-derived human MRP2 vesicles	NM_000392	5 mg/mL	0.5 mL
453804	HEK293-derived human BCRP vesicles	NM_004827	5 mg/mL	0.5 mL
453805	HEK293-derived human MRP3 vesicles	NM_003786	5 mg/mL	0.5 mL
453806	HEK293-derived human MRP4 vesicles	NM_005845	5 mg/mL	0.5 mL
459010	MRP/BCRP vesicle assay kit	N/A	–	Up to 200 assays
459011	BSEP vesicle assay kit	N/A	–	Up to 200 assays

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

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