



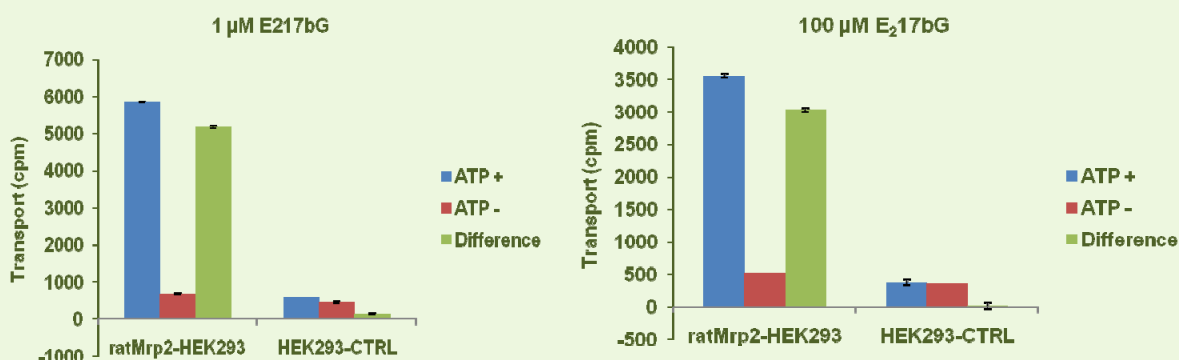
## rat Mrp2 Vesicular Transport Assay

The rat Mrp2 transporter (ABCC2) belongs to the family of ABC transporters. It is a full transporter localized in the apical membrane of polarized cells in various tissues and barriers, mainly in the intestine, liver and kidneys. Mrp2 has wide substrate specificity, transporting organic anions and drug conjugates (glutathions, glucuronides and sulfates), and is responsible for the excretion of drug metabolites through the bile and the urine.

A simple method for measuring rat Mrp2-mediated transport is the vesicular transport (VT) assay. SOLVO's rat Mrp2 vesicular transport assay uses membrane vesicles (ratMrp2-HEK293) isolated from transfected HEK293 cells stably expressing rat Mrp2 and control membrane vesicles (HEK293-CTRL) isolated from parental HEK293 cells, and employs estradiol <sup>3</sup>H-estradiol-17-β-D-glucuronide (E<sub>2</sub>17βG) as the probe substrate. E<sub>2</sub>17βG is a widely used probe substrate of human MRP2 and rat Mrp2. The interaction is detected as the modulation of the initial rate of E<sub>2</sub>17βG transport by rat Mrp2 into inside-out vesicles containing the transporter.

All assay validation steps were executed both at low (1 μM) and high (100 μM) E<sub>2</sub>17βG concentrations based on the human MRP2 studies of Zelcer et al (JBC, 2003). According to their observation many compounds stimulate the MRP2-mediated transport of E<sub>2</sub>17βG at low substrate concentrations. Such drug interactions could potentially affect the pharmacokinetic properties (e.g. oral bioavailability, biliary elimination) of drugs transported by MRP2. Similar stimulation was observed in the case of rat Mrp2-mediated E<sub>2</sub>17βG transport shown in this study (Figure 5).

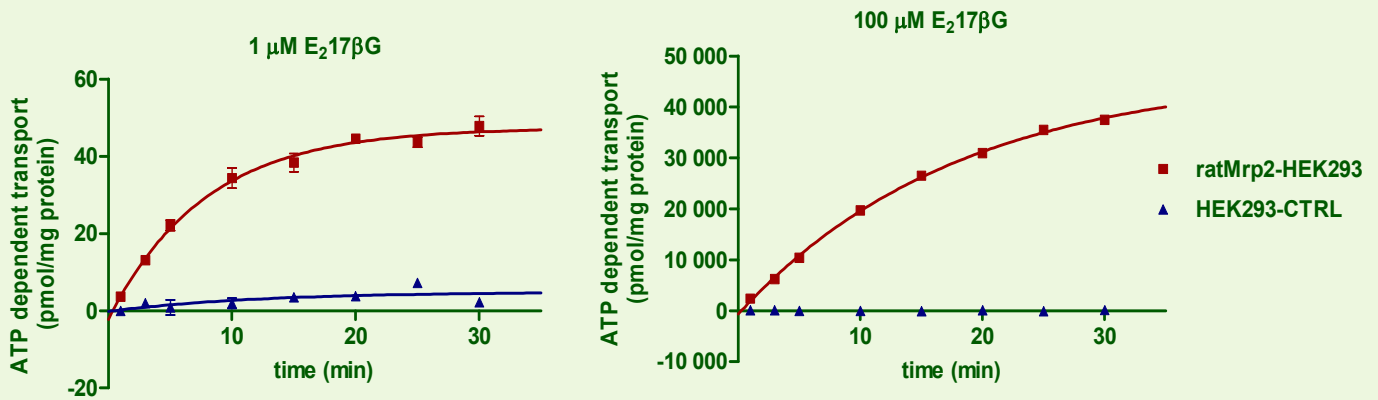
Rat Mrp2 transports E<sub>2</sub>17βG efficiently with negligible background transport in control vesicles (Figure 1). The transport was tested at 1 and 100 μM concentrations of E<sub>2</sub>17βG.



**Figure 1** – E<sub>2</sub>17βG transport (cpm values) into rat Mrp2 containing and control HEK293 vesicles. The experiment was performed in the presence of 1 and 100 μM E<sub>2</sub>17βG, at 37°C and 50 μg protein/well for 5 minutes.

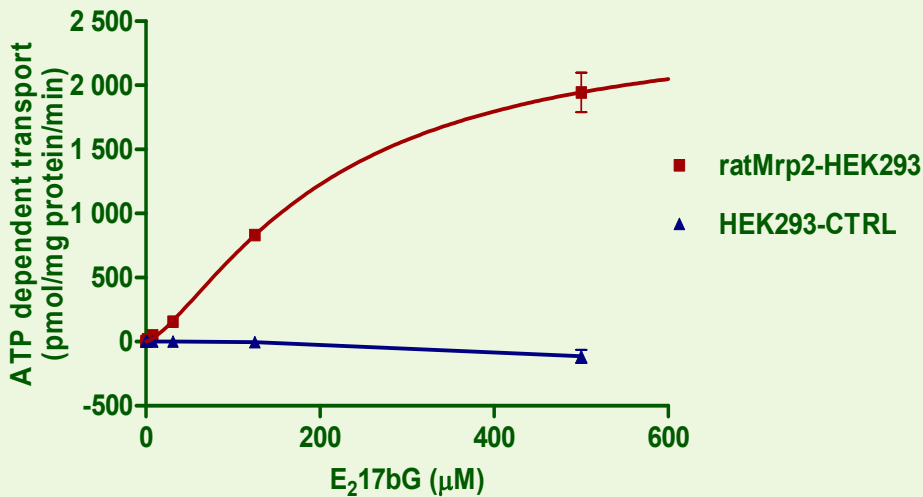


Time dependence of E<sub>2</sub>17βG transport by rat Mrp2 was assessed in a time interval of 30 minutes (Fig. 2.). An incubation time of 10 minutes was chosen in both cases for optimal signal-to-background ratio.



**Figure 2** – Uptake of E<sub>2</sub>17βG by rat Mrp2 containing and control HEK293 vesicles. The experiments were performed in the presence of 1 and 100 μM E<sub>2</sub>17βG at 37°C and 50 μg protein/well.

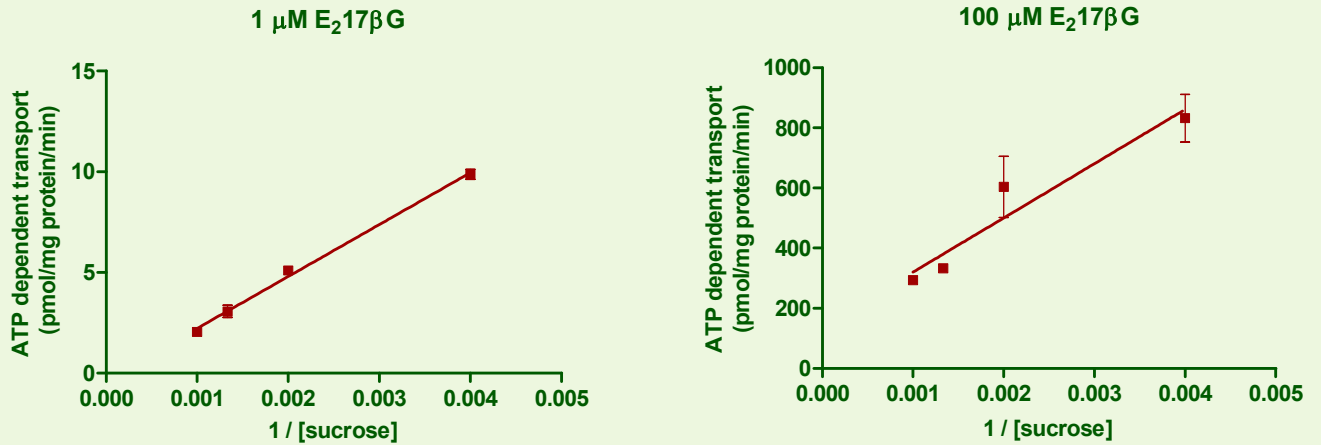
It was shown previously that the MRP2-mediated E<sub>2</sub>17βG transport does not follow Michaelis-Menten kinetics. This observation was also made in case of the rat Mrp2 as well, when expressed in HEK293 cells (this study). Control vesicles showed no concentration or ATP dependent accumulation of E<sub>2</sub>17βG.



**Figure 3** – rat Mrp2-mediated uptake of E<sub>2</sub>17βG at various substrate concentrations. Vesicles were incubated for 10 minutes, at 37°C and 50 μg protein/well.

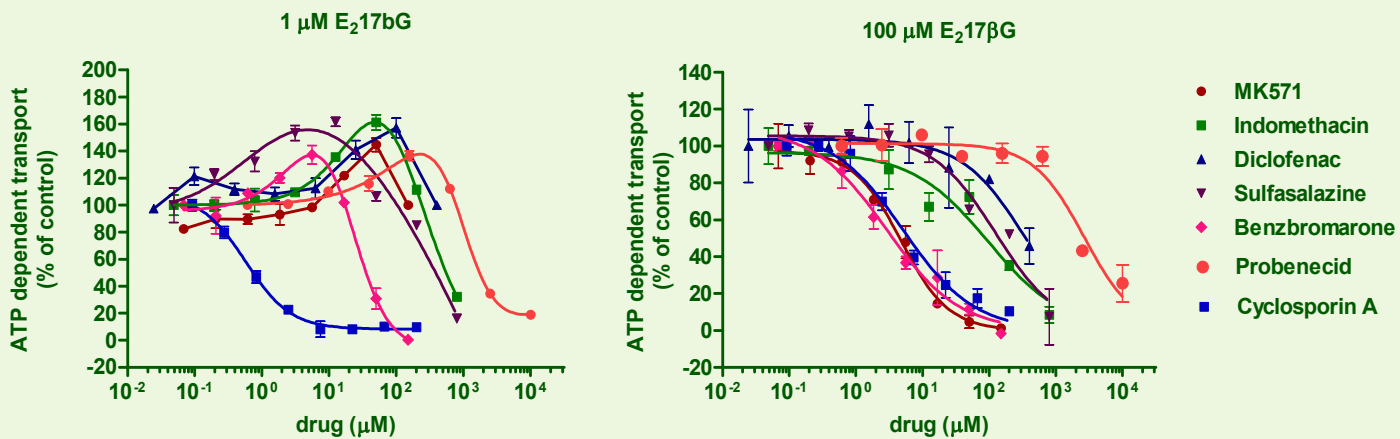


Applying different sucrose concentrations in the reaction buffer reveals a decreasing transport rate with increasing osmolarity; demonstrating that the transport is mediated by an active process (Fig. 4.).



**Figure 4** – Sucrose concentration dependence of rat Mrp2-mediated E<sub>2</sub>17βG transport

The inhibition curves determined both at low (1 μM) and high (100 μM) E<sub>2</sub>17βG concentrations are displayed in **Figure 5**.



**Figure 5** – Effect of known MRP2 interactors on the rat Mrp2-mediated E<sub>2</sub>17βG transport.



IC<sub>50</sub> values were determined in the ratMrp2-HEK293 VT assay for a set of known human MRP2 interactors using 100 μM E<sub>2</sub>17βG as the probe substrate (see **Table I**).

**Table I.** Comparison of IC<sub>50</sub> values generated in the ratMrp2-HEK293 vesicular transport inhibition assay to values measured in the human MRP2 vesicular transport assay.

MRP2 interactors	IC <sub>50</sub> (μM)	
	rat Mrp2	human MRP2
MK571	4.7	28.8
Sulfasalazine	124	77.4
Indomethacin	95.7	615
Diclofenac	327	NA
Benzbromarone	2.8	20.9
Probenecid	1500	8830
Cyclosporin A	5.14	2.0 (50% inh)