



MRP4 Vesicular Transport Assay

The MRP4 transporter (ABCC4) belongs to the family of ABC transporters. MRP4 is localized in the apical membrane of human and rat kidney proximal tubule epithelia and in the endothelial cells of brain capillaries. When transfected into MDCKII cell lines, MRP4 is localized in the basolateral membrane, and similar basolateral localization is observed in tubuloacinar cells of prostate and in human, rat and mouse hepatocytes. In summary, the membrane localization of MRP4 transporter in polarized cells is not properly elucidated yet.

By lacking one transmembrane domain compared to MRP1-3, MRP4 can transport and confer resistance to a variety of phosphorylated compounds, several endogenous organic anions and steroid conjugates, a variety of nucleoside analogues, as well as a wide range of xenobiotics.

SOLVO's MRP4 vesicular transport assay uses membrane vesicles isolated from transfectant LLC-PK1 cells stably expressing human MRP4, and employs ³H-dehydroepiandrosterone-sulfate (DHEAS) as the probe substrate. Human MRP4 transports DHEAS efficiently with negligible background transport in control vesicles (Figure 1).

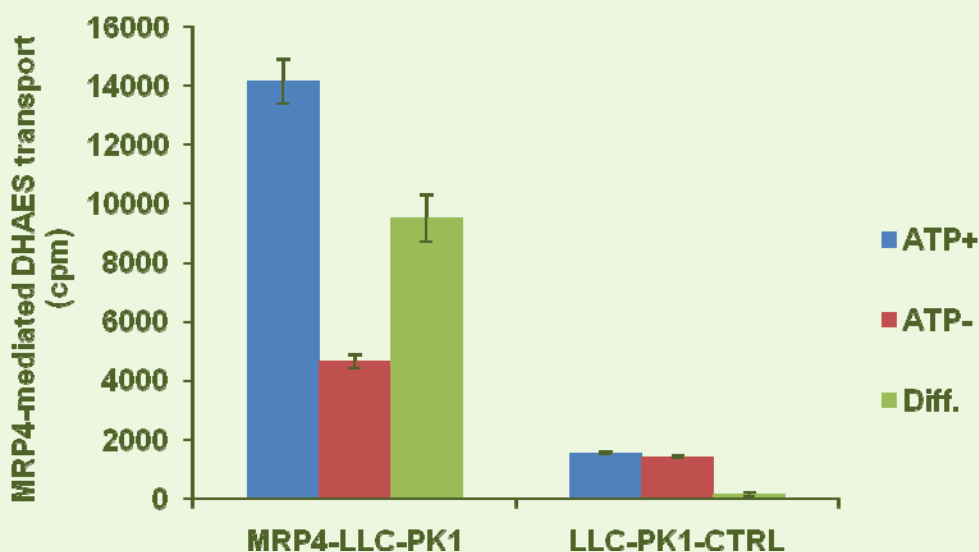


Figure 1. DHEAS transport (cpm values) into human MRP4 containing and control vesicles.



Time dependence of DHEAS transport by MRP4 was assessed in a time interval of 12 minutes (Fig. 2.). Saturation can be observed above 10 minutes in MRP4-LLC-PK1 vesicles, while background values are constant up to 15 minutes. An incubation time of 8 minutes was chosen for optimal signal-to-background ratio.

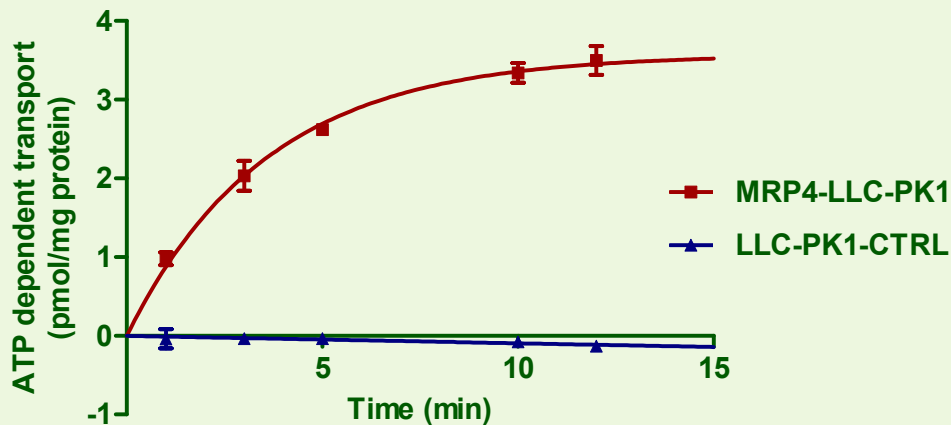


Figure 2 – Uptake of DHEAS by MRP4 containing and control LLC-PK1 vesicles. The experiment was performed in the presence of 0.02 μM 3H-DHEAS, at 37°C and 50 μg protein/well.

The transport is saturable and fits well to the Michaelis-Menten kinetics (Fig. 3.). KM was found to be around 15 μM and Vmax was 170 pmol/mg protein/min for MRP4. Control vesicles showed no or insignificant concentration and ATP dependent accumulation of DHEAS.

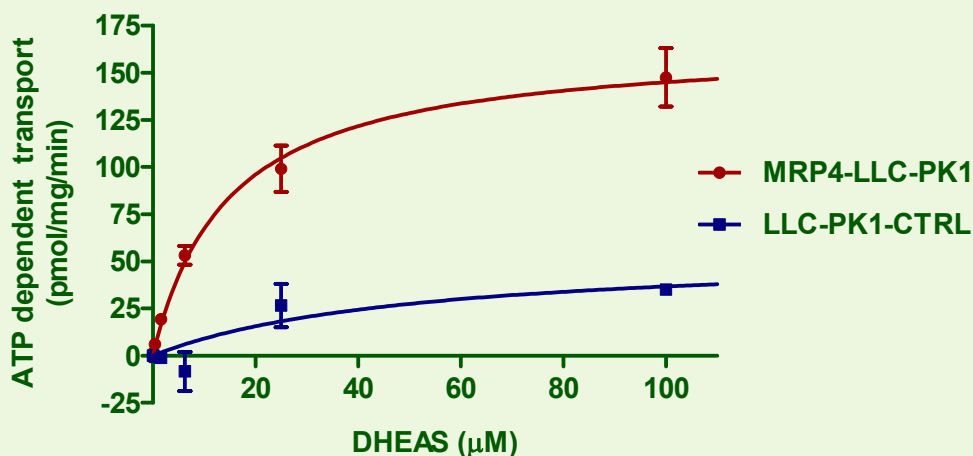


Figure 3 – MRP4-mediated uptake of DHEAS at various substrate concentrations. Vesicles were incubated for 8 minutes, at 37°C and 50 μg protein/well.



An evidence that the transport is mediated by an active process is the observation of decreasing transport rate with increasing osmolarity. This was achieved by applying different sucrose concentrations in the reaction buffer (Fig. 4.).

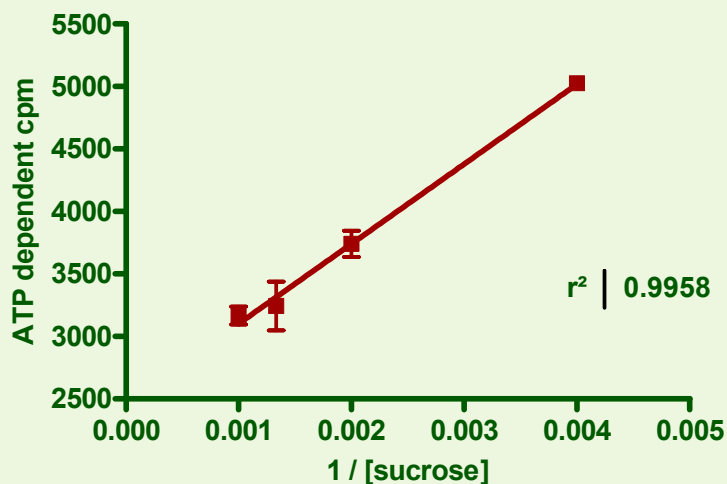


Figure 4 – Sucrose concentration dependence of MRP4-mediated DHEAS transport

IC₅₀ values were determined for a set of known MRP4 interactors using DHEAS as the probe substrate. Inhibition curves are displayed in **Figure 5.**, while **Table 1.** shows that the IC₅₀ values calculated are in good agreement with those obtained from the literature..

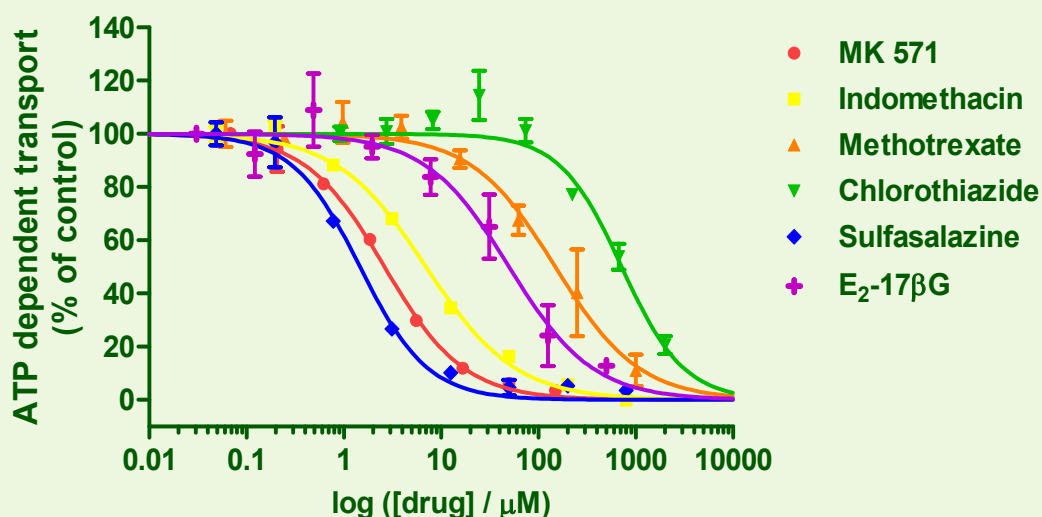


Figure 5 – Effect of known MRP4 interactors on the MRP4-mediated DHEAS transport.



Table 1. Comparison of IC₅₀ values generated in the human MRP4 vesicular transporter inhibition assay to values published by Russel F. *et al.* (Trends in Pharmacol. Sciences (2008) **29**:2000-2007).

MRP4 interactors	IC ₅₀ (μM)	
	MRP4	Literature
MK 571	2.39	2-10
Indomethacin	6.19	5-22
Methotrexate	167	220 (K _M)
Chlorothiazide	594.3	N/A
Sulfasalazine	1.23	N/A
E ₂ -17βG	43.1	30 (K _M)